

नेहरू बाल पुस्तकालय

युग-युग की कहानियां

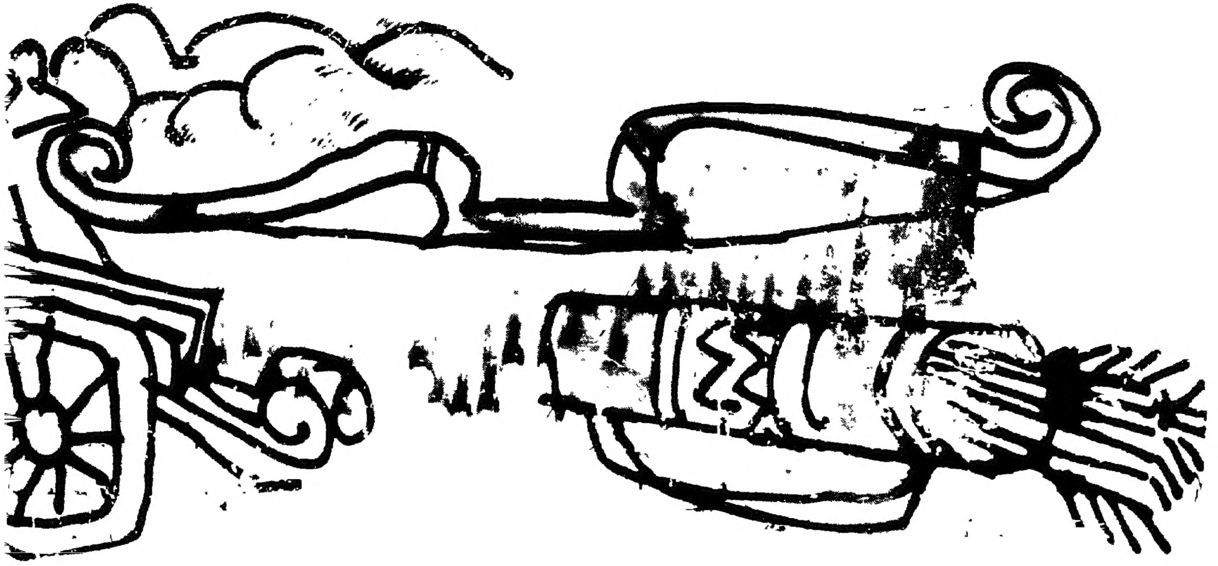
शांता रंगाचारी

चित्रांकन

पी. खेमराज

अनुवाद

मोहिनी राव





नेशनल बुक ट्रस्ट, इंडिया



ISBN 81-237-0017-2

पहला संस्करण 1972

तेइसवीं आवृत्ति 2002 (शक 1924)

Tales for All Times *(Hindi)*

रु. 12.00

निदेशक, नेशनल बुक ट्रस्ट, इंडिया, ए-5 ग्रीन पार्क
नयी दिल्ली-110016 द्वारा प्रकाशित



मृत्यु से संवाद

राजकुमारी सावित्री पिता के सामने खड़ी थी—सुंदर, सुकुमारी, लेकिन दृढ़-संकल्प। उसके चेहरे पर हठ था। उसके पिता राजा अश्वपति उसके हठीले स्वभाव को खूब जानते थे, लेकिन उसके सामने अपने को विवश पाते थे।

सावित्री ने पिता को याद दिलाया, "आपने कहा था कि मैं अपना पति स्वयं चुन सकती हूं। कहा था न? याद है न आपको, पिताजी? और अब आप अपने वचन से पीछे हट रहे हैं।"

पिता-पुत्री एक दूसरे से कटु वचन न कह बैठें, इस डर से नारद मुनि बात काटकर बीच ही में बोल उठे, "पुत्री, तुम्हारे पिता अपना वचन नहीं तोड़ रहे हैं। वह मुझसे सत्यवान के बारे में ही पूछ रहे थे जिससे तुम विवाह करना चाहती हो। मैं उनसे बात कर रहा था तो उन्होंने कहा सावित्री को बुलवा लें। वे चाहते थे कि मैं जो कुछ कहूं, वह तुम स्वयं अपने कानों से सुनो।"

"और आप क्या कह रहे थे?" सावित्री ने पूछा। सावित्री जानती थी कि

नारद के साथ बहुत सोच-समझ कर बात करनी चाहिए। सावित्री की बुद्धि बहुत तेज थी, लेकिन नारद उससे भी तेज थे। वह देवताओं और मनुष्यों दोनों के ही मित्र, सलाहकार और दूत थे। वह जो कुछ भी करते थे वह सब के भले के लिए ही होता। अंतिम परिणाम चाहे जितना सुखद हो, उनके काम प्रायः लोगों को अप्रिय लगते थे। नारद इतने चतुर थे कि कभी-कभी, बड़े-बड़े बुद्धिमान लोग भी हार जाते थे। नारद के लिए सावित्री के मन में बड़ा आदर था।

नारद ने कहा, ''सत्यवान जिससे तुम प्रेम करती हो, बड़े प्रतिष्ठित वंश का राजकुमार है और बहुत योग्य है। उसके नाम से ही उसके सच्चरित्र का पता चलता है। सत्यवान—अर्थात् जो केवल सत्य बोलता है। वह बुद्धिमान है, साहसी है, और पितृभक्त है। उसके अंधे वृद्ध पिता को उनके एक धोखेबाज संबंधी ने षड्यंत्र रचकर गद्दी से उतरवा दिया था। उनका जन्म तो हुआ था राजसिंहासन पर बैठने के लिए, लेकिन अब जंगल में रहते हैं और लकड़हारे का काम करते हैं बेचारे।''

''मैं सब कुछ जानती हूं।''

''तुम और भी बहुत कुछ जानती हो शायद,'' उसके मन की थाह पाने की कोशिश करते हुए नारद ने कहा।

''यह तो इस पर निर्भर करता है कि आप और क्या जानते हैं,'' सावित्री ने पैंतरा बदला

नारद ने मुस्कराकर मन ही मन सोचा, ''यह जरा-सी लड़की मुझे बनाने की कोशिश कर रही है!''

उन्होंने कहा, ''मुझे एक और बात मालूम है जो शायद तुम्हें नहीं मालूम। वही बात मैं अभी तुम्हारे पिता को बता रहा था।''

''यही बात न कि सत्यवान की जन्मपत्री में लिखा है कि आज से ठीक एक वर्ष बाद उनकी मृत्यु हो जायेगी?''

इस साहस से राजकुमारी ने ये शब्द कहे कि दोनों श्रोता दंग रह गये!

उसके पिता ने आवेश में आकर कहा, "फिर भी तुम उस युवक से विवाह करना चाहती हो?" उनकी आवाज कांप रही थी।

नारद ने सोचते हुए सावित्री की ओर देखा और पूछा, "तुमने कैसे यह मालूम किया, सावित्री? क्या तुम सत्यवान के मां-बाप से मिली थी?"

"हां," सावित्री ने उत्तर दिया। "उनकी मां बहुत ही धर्मपरायणा हैं। उन्होंने ही मुझे बताया। सत्यवान के जन्म के बाद जिन पंडितों ने उनकी जन्मपत्री बनायी थी, उन्होंने उनके माता-पिता को सावधान कर दिया था। सत्यवान इस बात को नहीं जानते, लेकिन उनके मां-बाप जानते हैं। इतने वर्षों तक उन्होंने इस रहस्य को छिपाये रखा और चिंता के भार को चुपचाप सहते रहे।"

नारद ने कहा, "एक बात बताओ, सावित्री। तुम समझती हो कि सत्यवान से विवाह करने पर तुम्हारा भविष्य कैसा होगा?"

"जी हां," सावित्री ने कहा।

"तुम्हें भय नहीं लगता?"

"दुखी अवश्य हूं," सावित्री ने गंभीरता से कहा, "लेकिन भय नहीं लगता। मैं यह नहीं मानती कि ब्राह्मण-पंडितों की गणना के अनुसार ग्रहों की स्थिति द्वारा जन्म और मृत्यु का निर्णय होता है। हम मनुष्य एक-दूसरे के जीवन को प्रभावित करते हैं। यदि मैं सत्यवान से विवाह करूंगी तो मेरे भाग्य का प्रभाव उसके जीवन पर पड़ेगा और कौन जाने क्या हो। हो सकता है कि होनी को रोका भी जा सके।"

सावित्री की बात सुनकर नारद मुनि ने उठते हुए कहा, "इस विवाह में अब रुकावट न डालो, अश्वपति। तिलक की तैयारी करो। सावित्री उन्हें प्रणाम करने आयी तो उन्होंने उसके सिर पर हाथ रखकर, गद्‌गद्-कंठ से कहा, "राजकुमारी, मैं केवल एक ब्रह्मचारी हूं, लेकिन मेरा आशीर्वाद तुम्हारे साथ है। भगवान से



प्राथना करूंगा कि तुम्हारा मंगल करें। मेरी कामना है बेटी, कि तुम्हारा साहस सदा सत्यवान की रक्षा करे।"

राजकुमारी उठकर अंदर जाने लगी तो अश्वपति उसकी ओर अपलक देखते रहे और ठंडी सांस भर कर बोले, "सावित्री को लड़का होना चाहिए था। स्त्रियों का इतना कुशाग्रबुद्धि होना अच्छा नहीं। कहीं इसकी प्रखर बुद्धि के कारण इसका अमंगल न हो।"

नारद ने स्नेहपूर्ण तिरस्कार से कहा, "इन मामलों में इतने पुराने विचार नहीं होने चाहिए, अश्वपति। जो भी हो, सावित्री की प्रखर बुद्धि लकड़हारे पति, अंधे ससुर और बेहद धार्मिक सास के साथ वन में रहने में उसकी अवश्य सहायता करेगी।"

लेकिन नारद की शंका गलत निकली। सावित्री-सत्यवान का विवाहित जीवन बहुत सुखी था। सावित्री को तो लगता कि जितना सुख वन में है उतना महल में नहीं।

बहुत सवेरे जब पक्षी चहचहाने लगते और गऊएं रंभा-रंभा कर अपने बछड़ों को बुलाने लगतीं, सावित्री की आंख खुल जाती। सास-ससुर उसको इतना लाड़-प्यार करते थे कि वह माता-पिता के वियोग को भी सह गयी। सास-ससुर ने ही उसे जप-तप का संयम-नियम समझाया। इस प्रकार सुख और स्वाधीनता के वातावरण में दिन निकलते गये और सावित्री किशोरी से युवती हो गयी।

सत्यवान की मृत्यु का भय हर समय उनके सुखी जीवन पर छाया रहता था। लेकिन सावित्री और उसके सास-ससुर के व्यवहार से यह जरा भी पता न चलता कि यह चिंता उन्हें घुन की तरह खाये जा रही है।

आखिर काल-दिवस आ पहुंचा। रोज की तरह सवेरा हुआ। पेड़ों पर चिड़ियां चहचहायीं, गऊएं रंभा-रंभा कर अपने बछड़ों को बुलाने लगीं। सत्यवान के माता-पिता चिंतित, उदास चेहरों से अपना-अपना काम-काज करने लगे। रह-रह कर वे प्रेम से इतने व्याकुल होकर पुत्र की ओर देखते कि सावित्री से सहा न जाता और वह अपना मुंह फेर लेती। सावित्री भी यंत्र के समान चुपचाप अपना काम कर रही थी। उसमें जैसे कुछ सोचने की शक्ति ही नहीं थी।

सत्यवान लकड़ी काटने के लिए जंगल जाने को तैयार हुआ तो सावित्री भी उठी।

"मैं आज आपके साथ चलूंगी। चलूं?" उसने पूछा।

"क्यों?" सत्यवान ने कहा, "आजकल धूप बहुत तेज होती है। और फिर तुम्हारी आदत है इधर-उधर घूमने निकल जाती हो और खो जाती हो।"

सावित्री ने मुस्कराने की चेष्टा करते हुए कहा, "आज मैं कहीं नहीं जाऊंगी। बस, बैठी-बैठी आपको देखती रहूंगी।"



अचानक सत्यवान की मां बोल उठीं, "उसको अपने साथ ले क्यों नहीं जाते, बेटा?"

सत्यवान ने हंसकर शरारत भरी आंखों से पूछा, "क्यों मां, आज सवेरे-सवेरे अपनी बहू से छुटकारा पाना चाहती हो?"

मां ने कहा, "नहीं, मैं अपने बेटे को समझाने की कोशिश कर रही हूं कि कभी-कभी पत्नी को दुलार करना चाहिए। इस बेचारी के लिए दिल बहलाने को यहां क्या रखा है।"

"तो मैं इसको दुलार करूं, मां? मैं तो इसे हीरे और लाल का हार देना चाहता हूं। उसकी जगह जंगल की सैर... रहने भी दो, इससे क्या दिल बहलेगा?"

सत्यवान की बात सुनकर सावित्री का दिल डूबने लगा, लेकिन अपनी व्यथा को छिपाकर, हंसकर उसने कहा, "ओह! हीरे-लाल का हार! खैर... आज तो जंगल की सैर करा दीजिए। लेकिन हार किसी न किसी दिन लेकर रहूंगी, छोड़ूंगी नहीं। याद रखिएगा। आपने वचन दिया है।"

हंसते-बोलते, एक-दूसरे से बहुमूल्य उपहारों का वायदा करते, लापरवाह प्रसन्न बच्चों की तरह दोनों वन की ओर चले। जब सत्यवान ने माता-पिता को प्रणाम किया तो उनकी आंखें उसके चेहरे से हट नहीं पायीं। वे उसके शरीर पर हाथ फेरते रह गये। सावित्री ने ऐसा बहाना किया मानो उसने कुछ देखा ही न हो। उसको डर था कि कहीं आंखों से आंसू न बहने लगें। वह बार-बार अपने को समझाती रही, "आज मुझे अपने सारे साहस और संयम की जरूरत है। अपने मित्रों, ब्राह्मणों और नारद मुनि के आशीर्वाद की जरूरत है। अब मेरा भाग्य मेरे ही हाथों में है।"

जंगल में पहुंच कर सत्यवान ने अच्छी तरह देख-भाल कर काटने के लिए एक पेड़ चुना, और उसके तने पर कुल्हाड़ी चलाने लगा। सावित्री चारों तरफ

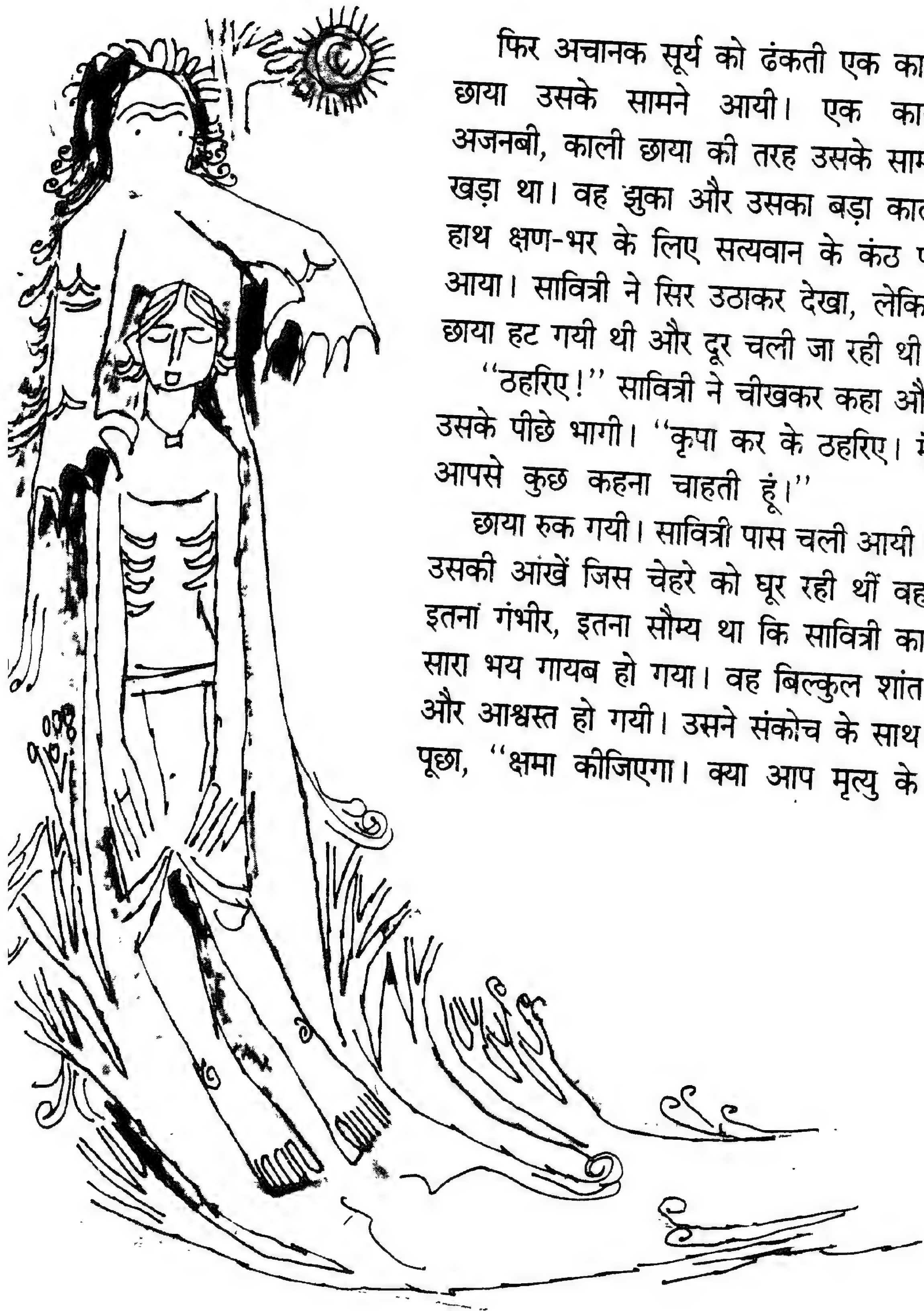


देखती रही कि कहीं से कोई सांप या बनैला पशु न आ जाए। वह सोच रही थी कि सांप या जंगली पशु के रूप में ही मृत्यु उसके पति पर वार करेगी। कुल्हाड़ी चलाते-चलाते अचानक सत्यवान ने अपना सिर थाम लिया और लड़खड़ाता हुआ पत्नी के निकट आया।

"ओह कितनी भयंकर पीड़ा हो रही है सिर में," इतना कहना था कि गिरकर वह मूर्च्छित हो गया।

सावित्री ने तुरंत पति का सिर अपनी गोद में रख लिया और इधर-इधर देखने लगी कि कहीं कोई है जिसे सहायता के लिए पुकारा जा सके? सत्यवान के मूर्च्छित होकर गिरने से सावित्री स्तब्ध होकर ऐसे बैठी रही मानों पत्थर की मूर्ति हो।





फिर अचानक सूर्य को ढंकती एक काली छाया उसके सामने आयी। एक काला अजनबी, काली छाया की तरह उसके सामने खड़ा था। वह झुका और उसका बड़ा काला हाथ क्षण-भर के लिए सत्यवान के कंठ पर आया। सावित्री ने सिर उठाकर देखा, लेकिन छाया हट गयी थी और दूर चली जा रही थी।

"ठहरिए!" सावित्री ने चीखकर कहा और उसके पीछे भागी। "कृपा कर के ठहरिए। मैं आपसे कुछ कहना चाहती हूं।"

छाया रुक गयी। सावित्री पास चली आयी। उसकी आंखें जिस चेहरे को घूर रही थीं वह इतना गंभीर, इतना सौम्य था कि सावित्री का सारा भय गायब हो गया। वह बिल्कुल शांत और आश्वस्त हो गयी। उसने संकोच के साथ पूछा, "क्षमा कीजिएगा। क्या आप मृत्यु के

देवता हैं? मैंने आपके बारे में बहुत कुछ सुना हूं,'' सावित्री बोलती रही, ''लेकिन अभी बहुत कुछ जानना चाहती हूं। एक बात पूछ सकती हूं? क्या आप हर एक के प्राणों को ले जाने स्वयं आते हैं?''

मृत्यु के देवता ने आगे चलते-चलते कहा, ''नहीं, मैं खास-खास लोगों के लिए ही आता हूं।''

''यदि सत्यवान खास लोगों में थे तो इतनी कम अवस्था में उनका जीवन क्यों ले लिया गया? और फिर उन्होंने कोई अपराध भी तो नहीं किया था।''

''मृत्यु दंड नहीं है,'' यमराज बोले।

सावित्री जल्दी-जल्दी कदम बढ़ाने लगी ताकि उस अजनबी के साथ कदम मिलाकर चल सके।

''मृत्यु अगर दंड नहीं है,'' उसने पूछा, ''तो किस जीवन का कब अंत होगा, इसका फैसला कौन करता है? किसी के जन्म के समय ही उसकी मृत्यु का फैसला कर लेना तो अन्याय है। और फिर इसमें कोई तर्क भी नहीं है।''



छाया ने रुककर कहा, "यह समझना आसान नहीं है। तुम वापस क्यों नहीं चली जाती, सावित्री? तुम मेरा पीछा क्यों कर रही हो?"

"मैं आपका पीछा नहीं कर रही हूं," सावित्री ने भोलेपन से कहा। "मैं तो अपने पति के पीछे-पीछे जा रही हूं।"

"लेकिन यह अब तुम्हारा पति नहीं है।

सावित्री ने धीरे से कहा, "लेकिन मैं तो सोचती हूं कि प्रेम जीवन और मृत्यु से परे की चीज है। हम कहते हैं कि किसी स्त्री और पुरुष का आपस में प्रेम हो जाना केवल एक दूसरे को पहचान लेना है। आप पहचान उसी को सकते हैं जिससे पहले परिचय रहा हो। मैंने तो सत्यवान को पहली बार देखते ही पहचान लिया था। हम लोग पहले से ही एक-दूसरे को जानते हैं। और ऐसा दोनों के पूर्व जन्म में ही हुआ होगा। अपने पूर्व जन्म में हम एक-दूसरे को जानते थे, इस कारण इस जन्म में हम एक-दूसरे को देखते ही पहचान गये। इसी कारण मैं सत्यवान को नहीं छोड़ सकती। हमारा जन्म-जन्म का साथ है। यदि आप इन्हें ले जायेंगे तो मुझे भी ले जाना पड़ेगा।"

मृत्यु के देवता यम कुछ हंसकर बोले, "तुम बहुत हठी हो। तुम्हारा तर्क सुनकर मुझको हंसी आती है। तुम्हारी कोई इच्छा है? सत्यवान के जीवन को छोड़कर कुछ भी मांगो। मैं दूंगा।"

सावित्री सोचने लगी।

सोचकर उसने कहा, "अच्छी बात है। आप तो जानते हैं कि मेरे ससुर को धोखा देकर उनकी गद्दी छीन ली गयी थी। मैं सोचती हूं कि इस अन्याय का प्रतिकार होना चाहिए। मुझे आशा है कि आप मुझसे सहमत होंगे।"

"तथास्तु," यम ने कहा, और जल्दी-जल्दी चलने लगे। कुछ दूर जाने के बाद उन्होंने मुड़कर देखा कि सावित्री अब भी उनके पीछे आ रही है।

उसके तलुए कांटों और कंकडों से छिलकर लहूलुहान हो गये थे।

मुस्कराकर उसने कहा, "आप बहुत तेज चलते हैं।"

यम ने उसकी ओर कठोर दृष्टि से देखकर पूछा, "तुम चाहती क्या हो, सावित्री। यदि तुम अपने पति का जीवन चाहती हो तो तुरंत इसका विचार छोड़ दो क्योंकि यह संभव नहीं है। यम न तो अपना वचन तोड़ता है और न किसी का जीवन लौटाता है। समझी?"

"ओह, यह बात है?" सावित्री ने बड़ी सोच में सिर हिला कर कहा। "बड़ी दिलचस्प बात है। लेकिन तब तो आपको पक्का विश्वास होगा कि आप जो कुछ करते हैं, ठीक करते हैं। है न?"

"इसमें ठीक और गलत का कोई प्रश्न नहीं।"

"अच्छा," सावित्री ने आश्चर्य से कहा, "कितनी अजीब बात है। बचपन से ही कूट-कूट कर मेरे दिमाग में यह बात भरी गयी है कि हमेशा वही काम करना चाहिए जो ठीक हो। गलत काम नहीं करना चाहिए। लेकिन शायद ठीक या गलत यह सब केवल हम मनुष्यों के लिए है, देवताओं के लिए नहीं।"

"लेकिन मृत्यु की बात अलग है।"

"कैसे? मुझको तो यही ठीक लगता है कि जो बात जीवन पर लागू है वही मृत्यु पर भी लागू होनी चाहिए।"

"तुम हर बात को इस तरह तोड़-मरोड़ देती हो कि वह तर्कपूर्ण लगने लगती है।"

"क्षमा चाहती हूं," सावित्री ने बड़े विनय से कहा। "मैं अपनी बात को दूसरी तरह कहूंगी। यदि सारा जीवन हम ठीक काम करने का प्रयत्न करते रहते हैं तो यह उचित ही है कि...।"

"ठहरो," यम ने कहा। यदि मैं तुम्हें एक वरदान और दूं तो मेरा पीछा करना छोड़ दोगी?"

सावित्री ने बड़े हर्ष से ताली बजाकर कहा, "तो क्या आप मुझको एक और

वरदान देंगे? कितने उदार और दयावान हैं आप!"

"लेकिन याद रखना। सत्यवान का जीवन मत मांगना।"

"नहीं, नहीं," सावित्री ने कहा। "जरा सोचने दीजिए। मैं अपने पहले वरदान में ही कुछ और मांगना चाहती थी।" भौंहें सिकोड़ कर वह कुछ सोचने लगी। फिर जैसे अचानक बात याद आ गयी हो, उसके कहा, "हां, अपने ससुर के लिए कुछ मांगना चाहती थी जिनका राजपाट आपने कृपा कर के वापस दिलवा दिया। वह अंधे हैं। एक अंधा आदमी राजपाट लेकर क्या करेगा? और प्रजा के लिए अंधा राजा किस काम का भला?"

यम ने मुस्कराकर कहा, "तुम्हारे ससुर की आंखें बिल्कुल ठीक हो जायेंगी।"

यह कहकर वह चलने लगे तो सावित्री ने फिर कहा, "मुझे बड़ी खुशी है कि मुझे अपने ससुर की याद हो आयी। अगर मुझे उनकी आंखों की बात याद न आ जाती तो जानते हैं उत्तेजना में मैं क्या मांग बैठती?"

"क्या?"

"अपने पिता और अपने ससुर के राज्यों के लिए वैभव और सुख। बात यह है कि अब दोनों राज्यों की उत्तराधिकारी मैं ही हूं। अब सोचती हूं कि अच्छा ही हुआ कि मैंने आपसे यह वरदान नहीं मांगा। इन राज्यों को भला वैभव और सुख क्यों दिया जाए? यह तो राजा का कर्तव्य है कि वह अपनी प्रजा को समृद्ध और सुखी बनाये। है न?"

"हां।"

"राजा की जिम्मेदारियां बहुत बड़ी हैं", सावित्री ने गंभीरता से सिर हिलाते हुए कहा। "महल में रहना, दरबार लगाना, कवियों और गायकों को सम्मानित करना, और कर वसूल करने के लिए कर्मचारियों को राज्य-भर में भेजना, इतना ही थोड़ा होता है राजा का काम? राजा को यह भी देखना होता है कि कानून का पालन उचित ढंग से हो, और उसके कर्मचारी प्रजा को न सताएं। उसको

पड़ोसियों के साथ शांति बनाये रखनी चाहिए, और यह देखना चाहिए कि उसके राज्य में कोई ऐसा तो नहीं है जिसके पास भोजन, कपड़ा या रहने की जगह नहीं है। इसमें भी जरूरी यह है कि लोगों को बोलने की आजादी हो, और वे राज्य-व्यवस्था की आलोचना निर्भय होकर कर सकें जिससे कि राजा स्वेच्छाचारी न बन जाये।"

इस बुद्धिमती राजकुमारी की बातें सुनकर यम मन ही मन उसकी प्रशंसा करते हुए बोले, "बिल्कुल ठीक कह रही हो तुम। न्याय और स्वाधीनता की परंपरा पुस्तकों में लिखे कानूनों से कहीं ज्यादा बड़ी है।"

सावित्री ने कहा, "और इसके लिए यह आवश्यक है कि राजाओं के वंश बिना किसी विघ्न-बाधा के चलते जायें, उत्तराधिकार का सिलसिला कहीं न टूटे। है न?"

"अवश्य," यम ने कहा। "अगर उत्तराधिकार के सिलसिले में गड़बड़ी हुई तो अराजकता फैलेगी, संबंधियों में आपस में युद्ध होगा।"

सावित्री ने अचानक मौन साध लिया। उसके चेहरे पर निराशा और उदासी छा गयी, उसके कंधे झुक गये और उसकी सुंदर आंखों से आंसू टपक पड़े।

यम ने आश्चर्य से पूछा, "क्या हुआ, सावित्री?"

"आप इतने बुद्धिमान हैं, सर्वज्ञ हैं," सावित्री ने ठंडी सांस भर कर कहा। "मुझे विश्वास है कि आपने मेरे मन की बात समझ ली होगी।"

यम भौंहें सिकोड़कर सोचने लगे। सावित्री क्या सोच रही है यह समझने की कोशिश करना वैसा ही था जैसे तूफान में हवा की दिशा का अनुमान लगाना।

सावित्री ने धीरे से कहा, "मैं सोच रही थी कि मेरे बाद इन दोनों राज्यों का कोई शासक नहीं होगा। मेरे पिता और मेरे ससुर के वंशों का क्या होगा? मेरी मृत्यु के बाद कितनी अराजकता फैलेगी, संबंधियों में युद्ध होंगे—यही शब्द थे न आपके? सड़कों पर रक्त की नदियां बहेंगी, नगर उजाड़ हो जायेंगे, फसलों को



काटनेवाला कोई न होगा, घर-घर से स्त्रियों और बच्चों का रोना सुनायी देगा...।''

''ठहरो,'' यम ने उसको रोक कर कहा। ''ऐसा नहीं होने पायेगा। मैं तुमको वरदान देता हूं। तुम्हारे सौ पुत्र होंगे और वे दोनों वंशों को चलायेंगे।''

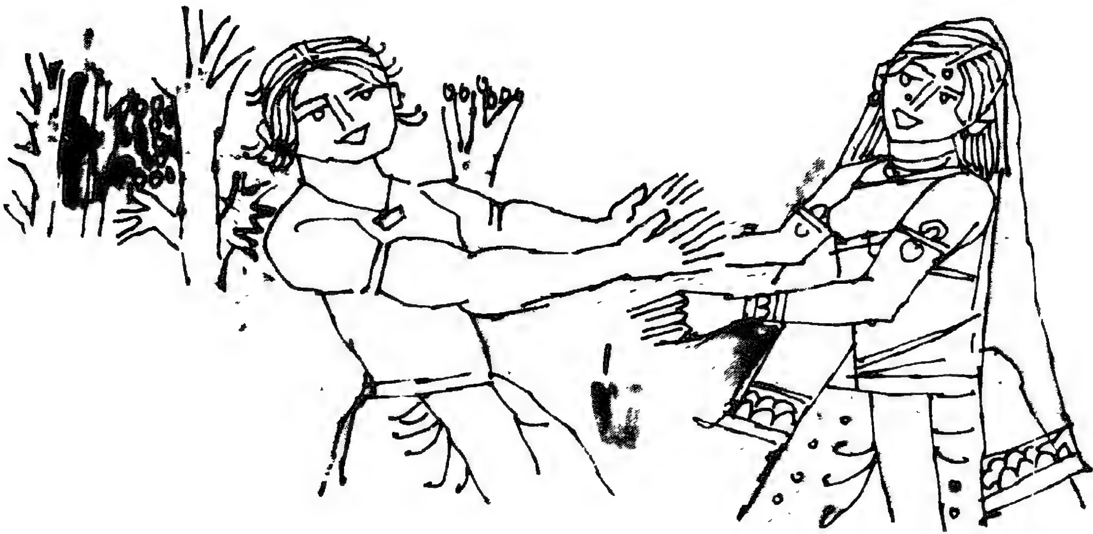
जैसे ही यम के मुख से यह शब्द निकले, सावित्री का सारा रूप ही मानों बदल गया। उसका चेहरा खुशी से चमकने लगा, झुके कंधे तन गये, ठंडी सांसें और आंसू मानों जादू से गायब हो गये। रानियों की-सी शान से सुंदर राजकुमारी यम के सामने सिर उठाये खड़ी थी।

उसने कहा, ''मुझे दुख है कि मेरे कारण आपको अपनी परंपरा तोड़नी पड़ेगी।''

''कौन-सी परंपरा?'' यम ने सतर्क होकर पूछा।

''यही कि यम कभी किसी का जीवन नहीं लौटाते। आपने मुझको सौ पुत्रों का वरदान दिया है न? यदि आप मेरे पति को ले गये तो मेरे पुत्र कैसे होंगे?

यम ने हार स्वीकार की। जब दोनों जल्दी-जल्दी जंगल में वापस जा रहे थे तो





यम ने कहा, "नारद ने मुझसे कहा था कि मैं सत्यवान को लेने स्वयं जाऊं। तभी मुझे समझ जाना चाहिए था कि इसमें नारद की कोई चाल है!"

कहने की आवश्यकता नहीं कि यमराज ने जो-जो वरदान सावित्री को दिए थे, सब पूरे हुए। सत्यवान आंखें मलता हुआ उठ बैठा मानों लंबी नींद से जगा हो। उसने सावित्री को बताया कि उसने एक विचित्र सपना देखा कि वह किसी काले अजनबी के साथ किसी लंबी यात्रा पर जा रहा है। सत्यवान के पिता को आंखें भी मिल गयीं और खोया हुआ राजपाट भी। सावित्री को हीरे और लाल का कीमती हार भी मिला।

भाग्य ने पलटा खाया। चारों तरफ खुशियां मनायी जाने लगीं। खाने-पीने, नाच-गाने की धूम मच गयी। जब धूम-धड़ाका खत्म हुआ और उत्साह ठंडा पड़ा तो एक दिन अश्वपति ने सावित्री को अलग बुलाकर पूछा, "मुझे समझ नहीं आता बेटी, कि यमराज से टक्कर लेने का साहस कैसे हुआ तुमको?"

सावित्री ने मुस्कराकर कहा, "पिताजी, सत्यवान की माताजी से जब मैं मिली तो उन्होंने मुझे उनकी जन्मपत्री और पंडितों की भविष्यवाणी के बारे में बताया। साथ ही उन्होंने यह भी कहा कि उनमें जो सबसे विद्वान पंडित थे उन्होंने बताया था कि मृत्यु से अधिक शक्तिशाली वस्तु सत्यवान की मृत्यु को टाल सकेगी। इसी से मुझे आशा बंधी और साहस हुआ। मेरे पास मृत्यु से अधिक शक्तिशाली चीज थी पिताजी—मेरा प्रेम।



सात दिनों का पहरा

प्राचीन काल में राजा लोग केवल शौक के लिए ही शिकार नहीं करते थे। बनैले पशुओं को खत्म करना भी उनका उद्देश्य था ताकि वे वानप्रस्थियों को परेशान न करें। जब कभी कोई राजा शिकार पर जाता तो दरबारियों, सैनिकों और सेवकों का बड़ा दल भी उसके साथ होता।

महाभारत के वीर नायक अभिमन्यु के पुत्र राजा परीक्षित एक दिन एक हिरन का पीछा कर रहे थे। निशाना बांधकर तीर जो उन्होंने चलाया तो हिरन घायल हो गया, लेकिन मरा नहीं। शिकार का नियम है कि जानवर की जान ले लो, मगर उसे पंगु बना कर मत छोड़ दो। किसी जानवर को घायल और पीड़ा से छटपटाता छोड़ देना अधर्म माना जाता था और अब भी माना जाता है। जब हिरन घायल हो गया तो राजा उसको मारकर कष्ट से छुटकारा दिलाने के उद्देश्य से उसका पीछा करते-करते जंगल के बिल्कुल भीतर पहुंच गये। उनके साथी कहीं पीछे छूट गये थे। राजा थक गये थे। उनको बड़े जोरों से भूख और प्यास लग रही



थी। लेकिन उन्होंने संकल्प कर रखा था कि जब तक हिरन को पीड़ा से छुटकारा नहीं दिला देंगे, वापस नहीं लौटेंगे।

अचानक राजा ने देखा कि पेड़ों के बीच एक खाली स्थान है। वहां एक वृद्ध ब्राह्मण गऊओं को सानी-पानी दे रहे थे। राजा ने उनके पास जाकर पूछा, "ब्राह्मण देवता, मैं अभिमन्यु का पुत्र और इस राज्य का शासक हूं। मैं एक घायल हिरन की तलाश में हूं। वह इस ओर तो नहीं आया? आपने तो नहीं देखा?"

ब्राह्मण सन्यासी का नाम शमीक था। संयोग से वह उनके मौन रहने का दिन था। उन्होंने राजा के प्रश्न का उत्तर नहीं दिया। ब्राह्मण के उत्तर न देने पर राजा को पहले तो आश्चर्य हुआ, फिर उन्होंने सोचा कि शायद वृद्ध ब्राह्मण को सुनायी नहीं देता, और उन्होंने ऊंची आवाज में फिर अपना प्रश्न दोहराया। शमीक राजा की ओर देखते रहे लेकिन इस बार भी उत्तर नहीं दिया। राजा ने इतनी देर में यह तो जान लिया था कि सन्यासी बहरे नहीं हैं, क्योंकि इसी बीच एक गाय ने दूध दुहने की बाल्टी को लात मारी और उसकी आवाज सुनकर ब्राह्मण ने फुर्ती से बाल्टी को थाम लिया और उसको उलटने से बचा लिया।

अब तो राजा को बहुत क्रोध आया। उन्होंने समझा कि ब्राह्मण बहुत धृष्ट है। एक सन्यासी की यह मजाल कि राजा के प्रश्न का उत्तर न दे? राजा ने चीखकर कहा कि यदि उन्होंने उसके प्रश्न का उत्तर न दिया तो इसका परिणाम अच्छा नहीं होगा। फिर भी सन्यासी केवल राजा की ओर दुखभरी दृष्टि से ताकते रहे। बोले कुछ भी नहीं।

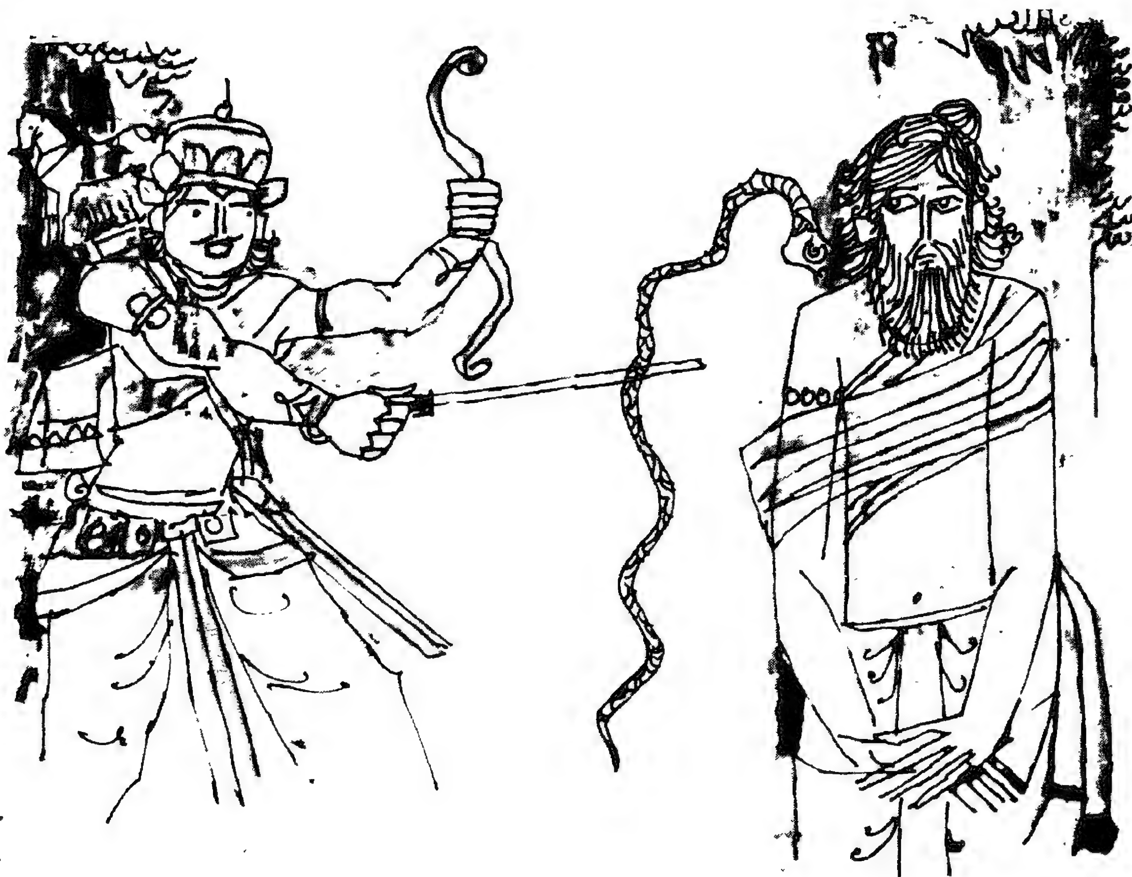
क्रोध में राजा आपे से बाहर हो रहे थे। उन्होंने इधर-उधर देखा कि किस

तरह उस धृष्ट ब्राह्मण को अपमानित करें। अचानक उनकी दृष्टि पास ही पड़े एक मरे हुए सांप पर पड़ी। तुरंत ही शाही तलवार म्यान से निकली और बिजली की तरह लपक कर मरे हुए सांप को नोक से उठा लिया। दूसरे ही क्षण सांप हवा में उछला और ब्राह्मण के गले में जा लिपटा।

राजा हंसे और इस प्रतीक्षा में खड़े रहे कि ब्राह्मण शमीक कुछ कहेंगे, शायद शाप भी दे दें।

लेकिन शमीक ने कुछ नहीं कहा। उनके चेहरे पर दुख का भाव भी उसी प्रकार बना रहा। राजा लज्जित होकर लौट पड़े।

लेकिन एक तीसरा व्यक्ति भी वहां उपस्थित था जो चुपचाप खड़ा यह सब कुछ देख रहा था। वह थे कृश, शमीक के पुत्र शृंगी के मित्र। लेकिन राजा या शमीक दोनों में से किसी को यह पता नहीं था। राजा के लौटने के बाद कृश शृंगी को यह समाचार देने भागे।





आखिर जब श्रृंगी मिले तो कृश ने उनसे पूछा, "तुमने उस दिन कहा था कि जंगल-वासियों के लिए सब से पहले भगवान हैं और फिर राजा। कहा था न?"

श्रृंगी दुर्लभ जड़ी-बूटियां जमा किया करते थे। अपना काम करते हुए उन्होंने अनमने भाव से कहा, "हां, कहा तो था।"

"यदि राजा हमारी परवाह न करे, तो हम उसके स्वामीभक्त क्यों हों?"

श्रृंगी ने पूछा, "तुम राजा परीक्षित की ही बात कर रहे हो न? वह वानप्रस्थियों का कभी अपमान नहीं करेंगे। क्यों करेंगे भला?"

श्रृंगी के मित्र ने, जो कुछ-कुछ शरारती थे, चालाकी से कहा, "मान लो मैं तुमसे यह कहूं कि मैंने स्वयं अपनी आंखों से देखा, तो?"

"तो भी मैं तुम्हारा विश्वास नहीं करूंगा," श्रृंगी ने तुरंत जवाब दिया।

"अगर तुम अपनी आंखों से इसका प्रमाण देखो तो?"

श्रृंगी ने खीझकर कहा, "कैसा प्रमाण? पहेलियां क्यों बुझा रहे हो? साफ-साफ क्यों नही कहते क्या बात है? तब शायद मैं उस किस्से को समझ सकूं जो तुम सुनाना चाह रहे हो।"

"यह कोई किस्सा-कहानी नहीं, सच बात है," श्रृंगी के मित्र ने उनका हाथ पकड़कर जंगल की ओर खींचते हुए कहा। वे दोनों वहां पहुंचे जहां शमीक समाधि लगाये बैठे थे। मरा हुआ सांप अभी तक उनके गले में लिपटा हुआ था।

"अरे! पिताजी के गले में मरा सांप लिपटा है!" यह कहते हुए श्रृंगी आगे की ओर लपके। लेकिन उनके मित्र ने उन्हें पीछे खींच लिया।

"हां, यह मरा हुआ सांप ही है", उन्होंने व्यंग से कहा। "हमारे महाराज, हमारे प्रभु और कृपालु रक्षक राजा परीक्षित ने इसे तुम्हारे पिताजी के गले में



डाला। मैंने स्वयं देखा। और जानते हो तुम्हारे पिता का अपराध क्या था जिसके लिए उन्हें यह दंड दिया गया? क्योंकि राजा ने किसी घायल हिरन के बारे में कुछ पूछा और तुम्हारे पिता ने उत्तर नहीं दिया। राजा उनके ऊपर खूब बिगड़े—मैंने स्वयं सुना। उसके बाद अपनी तलवार की नोक से इस मरे सांप को उठाकर तुम्हारे पिता के गले में डाल दिया। लेकिन मानना पड़ेगा—क्या हाथ की सफाई थी, वाह! ऐसा अचूक निशाना साधा कि सांप ठीक-ठीक तुम्हारे पिता के गले में आ लिपटा! जरा भी चूक नहीं हुई। मैंने स्वयं अपनी आंखों से देखा।''

''लेकिन,'' शृंगी ने चकित होकर पूछा, ''तुमने आगे बढ़कर महाराज को बताया क्यों नहीं कि आज पिताजी के मौन रहने का दिन है?''

''मेरा दिमाग थोड़ा ही खराब था?'' कृश ने मुंह बनाकर उत्तर दिया। ''महाराज बड़े क्रोध में थे उस समय और हाथ में नंगी तलवार थी? तलवार मेरी ही गर्दन पर चल जाती तो?''

शृंगी ने प्रेम और गर्व से अपने वृद्ध पिता की ओर देखा। मरा सांप देखकर उनको क्रोध आ रहा था।

''महाराज ने मेरे पिता का अपमान करके बहुत बुरा किया,'' उन्होंने कहा। ''क्या वह इनके मुख पर यह तेज नहीं देख सके? इनकी आंखों में गरिमा नहीं देख सके?''

''राजा लोग यह सब कुछ नहीं देखते,'' राजाओं के बारे में अपनी सम्मति को इस संक्षिप्त उत्तर में बता दिया कृश ने।

पिता के दुबले-सूखे शरीर पर राजा के अपमानजनक आचरण के प्रमाण को शृंगी जितना ही देखते, उनका क्रोध उतना ही बढ़ता जाता। आखिर उनसे अब और अधिक नहीं सहा गया और उनका सारा क्रोध शाप बनकर उनके मुंह से फूट पड़ा, "भले ही राजा हो, लेकिन वह नीच है जिसने मेरे महान पिता के गले में मरा हुआ सांप डाला। उसने मेरे पिता का ही अपमान नहीं किया, इन सारे वानप्रस्थियों का अपमान किया है जिन्होंने राजा को कभी कोई हानि नहीं



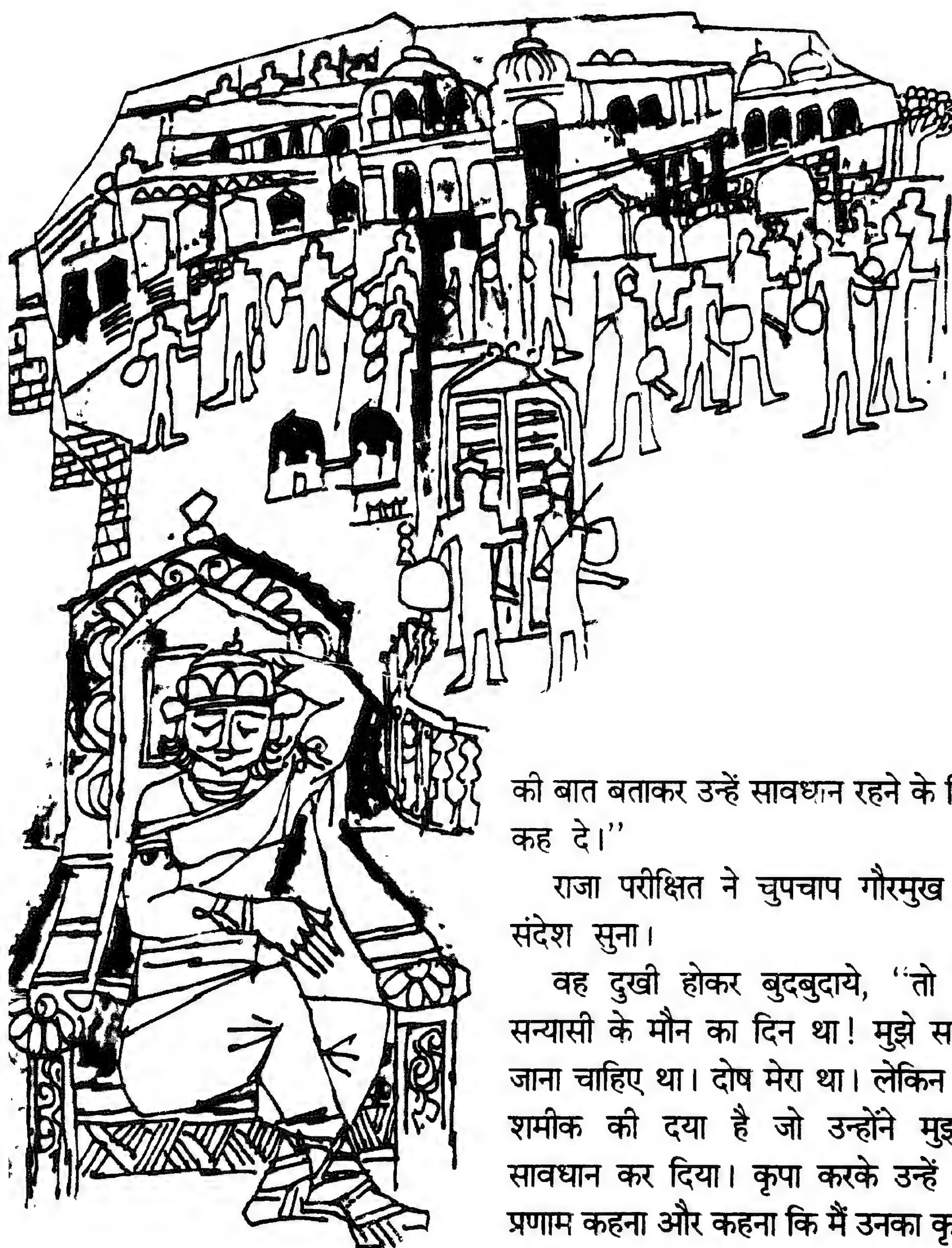
पहुंचायी। मैं राजा को शाप देता हूं। कुरु वंश के उज्ज्वल नाम को कलंकित करने वाले इस अहंकारी राजा की, आज से सात रोज के अंदर, सर्पराज तक्षक के काटने से मृत्यु हो जायेगी।''

इस भयानक शाप के शब्द शृंगी के मुख से निकले ही थे कि उनके पिता ने विचलित होकर अपनी समाधि तोड़ दी और भय से अपने पुत्र के मुंह की ओर देखने लगे। उनके बेटे ने क्रोध में आकर जो कुछ कह डाला था उससे मानों उन पर वज्रपात-सा हुआ। अपने मौन को तोड़ते हुए, आहत स्वर में बोले, ''शृंगी, मेरे बेटे, तुमने यह क्या कर डाला? तुमने नेक राजा परीक्षित को शाप दे डाला जिन्होंने सदा हमारी रक्षा की है, जिनकी कृपा से ही हम वन में शांति के साथ निर्भय होकर रह रहे हैं। तुमने किस कारण ऐसा पागलपन किया? क्या सन्यासियों का यही धर्म है? भगवान ही जानता है कि तुम्हारे क्रोध को वश में न रख पाने के कारण कैसा संकट आयेगा, कैसी अराजकता फैलेगी। तुमने राजा परीक्षित को शाप नहीं दिया, सारे राज्य को शाप दे डाला है, शृंगी। किसी भी दशा में ऐसा करना बहुत ही बुरा होता। राजा परीक्षित के साथ ऐसा करना तो और भी बुरा है क्योंकि वह दंड के भागी नहीं हैं।''

शृंगी का क्रोध उतर चुका था। पिता की बात सुनकर वह पश्चात्ताप से सिर झुकाये खड़े रहे, फिर रोने लगे।

''दुख की बात है कि तुम शाप को वापस भी नहीं ले सकते,'' शमीक ने दुख से विकल होकर कहा। ''तुमने अपने अनुशासन और अपनी विद्वत्ता से यह वरदान पा लिया है कि तुम्हारे मुख से निकली हर बात सच होकर रहेगी। यह जान कर ही मैंने तुम्हें बार-बार समझाया था बेटे, कि बोलने से पहले सौ बार सोच लिया करो।''

वृद्ध शमीक विचार में डूबे बैठे रहे। फिर अचानक उठकर बोले, ''गौरमुख को मेरे पास भेज दो। उसे मैं तुरंत राजमहल भेजूंगा कि वह महाराज को शाप



की बात बताकर उन्हें सावधान रहने के लिए कह दे।"

राजा परीक्षित ने चुपचाप गौरमुख का संदेश सुना।

वह दुखी होकर बुदबुदाये, "तो वह सन्यासी के मौन का दिन था! मुझे समझ जाना चाहिए था। दोष मेरा था। लेकिन यह शमीक की दया है जो उन्होंने मुझको सावधान कर दिया। कृपा करके उन्हें मेरा प्रणाम कहना और कहना कि मैं उनका कृतज्ञ हूं।"

यह कह कर गौरमुख को तो उन्होंने तुरंत विदा कर दिया और सलाह के लिए अपने मंत्रिमंडल को बुला भेजा। मंत्रियों के आने से पहले राजा ने कश्यप को संदेश भिजवाया कि वह तुरंत महल में आ जायें। कश्यप ब्राह्मण थे और सबसे अधिक विषैले सांप के काटे का भी इलाज कर सकते थे।

मंत्रिमंडल के निर्णय के बाद शिल्पी और राज-मिस्त्री महल में बुलवाये गये और रात ही रात में एक अजीब-सी इमारत खड़ी कर दी गयी—बस, एक ऊंचे खंभे पर एक बड़ा कमरा। इसी कमरे में राजा रहने लगे—खंभे के नीचे और कमरे के बाहर सशस्त्र संतरी खड़े थे। उनको कठोर निर्देश था कि कोई कीड़ा भी कमरे के अंदर न घुसने पाये। परिवार के लोगों और मंत्रियों को छोड़, राजा के पास जाने की अनुमति किसी को नहीं थी।

जब कश्यप राजा का आदेश पाकर जल्दी-जल्दी महल की ओर जा रहे थे तो रास्ते में एक बूढ़ा ब्राह्मण बैठा दिखा। वह बहुत ही दुखी लग रहा था।

कश्यप ने पूछा, ''क्या हुआ, भाई? सड़क के किनारे इस तरह दुखी क्यों बैठे हो?''

ब्राह्मण ने कहा, ''वही कारण है जो आपको इस सड़क पर लिये जा रहा है।''



"वही कारण है?" चकित होकर कश्यप ने पूछा, "पर मैं तो महाराज के दर्शनों के लिए जा रहा हूं। क्या तुम भी वहीं जा रहे हो?"

"हां"।

"राजा को धमकी दी गयी है कि वे नागराज तक्षक द्वारा काटे जायेंगे। उन्हीं की चिकित्सा के लिए मुझे बुलवाया गया है। लेकिन भला आपको क्या काम वहां ?"

"मैं उनको मारने जा रहा हूं।" कहते ही ब्राह्मण ने अपना असली रूप धारण कर लिया। असल में वह सर्पराज तक्षक था।

"यह तो अजीब स्थिति है," कश्यप ने कहा। "तुम उन्हें मारने जा रहे हो और मैं जिलाने। हम साथ-साथ चलें या अलग-अलग?"

तक्षक ने पूछा, "क्या आपको विश्वास है कि आप मेरे विष से राजा को बचा सकेंगे?"

"हां," कश्यप ने बिना किसी संकोच के कहा।

"साबित कीजिए," तक्षक ने चुनौती दी। "मैं इस पौधे को डसता हूं। देखें आप इसे फिर से जिला सकते हैं या नहीं।"

यह कहकर सर्पराज ने अपना मुंह खोला और पौधे को डसकर उसकी जड़ में गहराई तक अपना विष फैला दिया। कुछ क्षणों में पौधा इस प्रकार भस्म हो गया मानों अंदर की आग से जल गया हो। अपने काम से बहुत संतुष्ट होकर तक्षक ने कश्यप से कहा, "चलिए, अब अपनी शक्ति आजमाइए।"

कश्यप ने मुट्ठी-भर राख उठा ली, और प्रार्थना की मुद्रा में आंखें मूंद कर, उसमें हल्की-सी फूंक मारी। फिर राख को उसी जगह गाड़ दिया जहां से उसे उठाया था। कुछ ही देर में वहां हरा अंकुर निकल आया, फिर उसमें दो हरी पत्तियां फूट निकलीं। फिर हरा तना बढ़ने लगा, उसमें नयी-नयी पत्तियां निकलने लगीं और थोड़ी ही देर में पौधा वैसा ही हो गया जैसा पहले था।

तक्षक पहले तो घोर आश्चर्य से यह सब कुछ देखता रहा, फिर हार मान गया। इस प्रकार की चीज उसके लिए नयी नहीं थी। वह कई साधु-सन्यासियों से मिलता रहता था जो ऐसे करिश्मे करते थे और प्रार्थना के बल पर ही चमत्कार कर दिखाते थे।

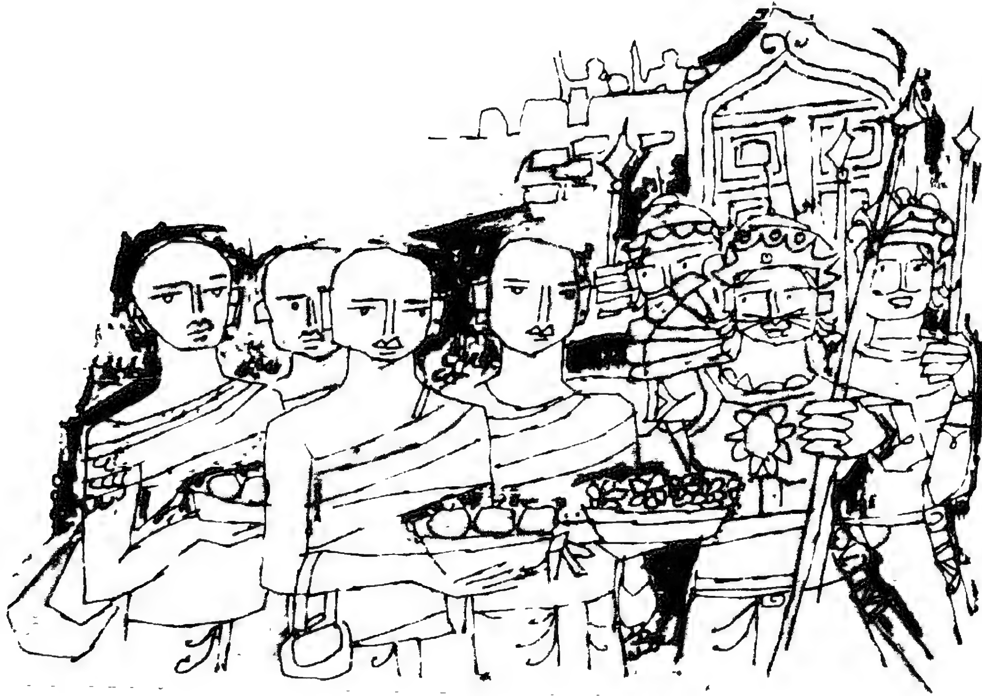
तक्षक ने कश्यप से कहा, "मैं आपकी शक्ति मानता हूं। लेकिन राजा परीक्षित के मामले में इसका प्रयोग मत कीजिएगा। इसका कारण है।"

"क्या कारण है?" कश्यप ने पूछा।

"पहले मैं आपसे एक प्रश्न पूछता हूं," उसने कश्यप से कहा। "क्या आप किसी की होनी में दखल देना उचित समझेंगे?"

"नहीं, उचित तो नहीं समझता। लेकिन यह होनी नहीं, अभिशाप है जो होनी में दखल दे रहा है।"

"नहीं, आपका विचार गलत है," तक्षक ने कहा। "मैं राजा को समय से पहले मारने के लिए नहीं जा रहा हूं। मैं तो मृत्यु को बुलाने जा रहा हूं क्योंकि उनके भाग्य में यही लिखा है। मुझे यमराज ने भेजा है—जन्म और मृत्यु के लेखे के अनुसार शृंगी ने जो कुछ कह डाला वह शाप नहीं, भविष्यवाणी थी।"



कश्यप गहरे विचार में डूबे खड़े रहे। "तो क्या राजा परीक्षित के भाग्य में लिखा है कि वे सात दिन के भीतर ही मर जायेंगे?" उन्होंने पूछा, "श्रृंगी ने शाप न दिया होता तो भी क्या यही होता?"

"देवताओं ने यही उनके भाग्य में लिखा था। मैं तो केवल मृत्यु-देवता का दूत हूं, समय से पहले ही किसी को समाप्त कर देने का साधन नहीं।"

"तब तो मैं इसमें हस्तक्षेप नहीं करूंगा," यह कहकर कश्यप अपने आश्रम वापस चले गये।

तक्षक बड़ी देर तक राजा के कक्ष में घुसने की तरकीब सोचता रहा। श्रृंगी की भविष्यवाणी के सातवें दिन उसे एक तरकीब सूझी। उसने झटपट अपने कुछ सर्प मित्रों को बुला भेजा और उन्हें आदेश दिये।

उसने कहा, "यह काम धोखे से ही किया जा सकता है। राजा की रक्षा का इंतज़ाम बहुत पक्का है।"

उस समय राजा परीक्षित, उनके परिवार के लोग और उनके दरबारी इस बात की खुशी मना रहे थे कि छह दिन बिना किसी संकट के टल गये। सातवां दिन भी समाप्त होनेवाला था। सूर्यास्त के साथ-साथ शाप का भी अंत हो जायेगा, और दिन डूबने को कुल एक घंटा बाकी था।

उस दिन काफी संध्या बीते कुछ साधु-सन्यासी खंभे के नीचे खड़े दिखायी दिये। एक ने प्रहरियों से कहा, "हम फल-फूल की भेंट लेकर महाराज को आशीर्वाद देने बहुत दूर से आये हैं।"

प्रहरियों ने सन्यासियों के कपड़ों और फल-फूल की टोकरी की अच्छी तरह तलाशी ली। जब संदेह की कोई बात न दिखायी दी तो उन्हें राजा के पास जाने दिया। उन्होंने सोचा, शाम का समय करीब-करीब बीत चुका है, राजा को अगर ये सन्यासी आशीर्वाद देने गये तो कोई हर्ज नहीं। सन्यासियों ने फल-फूल की टोकरी राजा को भेंट की, उन्हें आशीर्वाद दिया और बाहर चले गये। जंगल में



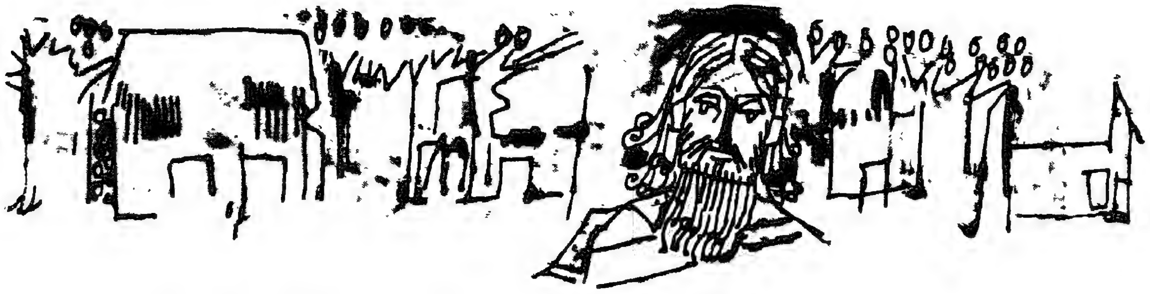
पहुंच कर सन्यासियों ने अपना सर्प का असली रूप धारण कर लिया और जंगल की हरियाली में गायब हो गये।

सूर्यास्त का समय हुआ। राजा के कक्ष में खुशियां मनायी जा रही थीं। बस थोड़ी देर और, फिर शाप का समय निकल जायेगा, और राजा को नयी आयु मिलेगी।

राजा ने अपने परिवार और मंत्रियों को बुलाकर कहा, "सूरज डूब रहा है। आओ, हमारे साथ ये स्वादिष्ट फल खाओ जो कृपालु सन्यासी दे गये हैं।"

दरबारियों ने हाथ बढ़ाकर अपनी-अपनी पसंद का फल उठा लिया। राजा का हाथ एक रसीले आम पर पड़ा। उन्होंने आम चूसा, बड़ा ही स्वादिष्ट था। कुछ देर बाद उन्होंने देखा कि उसकी गुठली में एक नन्हा सा कीड़ा है। यह कोई आश्चर्य की बात नहीं थी। प्रायः रसभरे आमों की गुठालियों में कीड़े निकल आते थे। राजा ने खिड़की से बाहर अस्त होते हुए सूर्य को देखा और हंसकर उस नन्हे काले कीड़े को लक्ष्य करके बोले, "अब तक्षक के आने का समय नहीं रहा। बोलो नन्हे कीड़े, तुम जानते हो कि तुम्हारा राजा अपने काम में असफल क्यों हो गया? लेकिन तुम्हें भला क्या मालूम? इसका उत्तर तो तक्षक ही दे सकता है।"

इतना कहना था कि राजा की भय से फैली आंखों ने देखा कि वह नन्हा-सा काला कीड़ा अचानक बढ़कर एक बड़ा शानदार नाग बन गया। जैसे-ही सूर्य पश्चिम में डूबा, तक्षक ने अपना विशाल फन फैलाया और राजा परीक्षित को तुरंत डस लिया।



उपमन्यु ने सबक सीखा

बहुत, बहुत दिन हुए, धौम्य नाम के एक ऋषि थे। उनके आश्रम में अनेक बालक पढ़ा करते थे। उनमें से एक का नाम था उपमन्यु। उपमन्यु और दूसरे बालक गुरुजी के साथ आश्रम में ही रहते थे और शिक्षा ग्रहण करते थे।

आश्रम के नियम बड़े ही कठोर थे। आज्ञाकारिता के बारे में तो बड़ा ही कठिन नियम था। गुरुजी के प्रत्येक आदेश का पालन आंखें बंद करके करना होता था। प्रश्न या किसी प्रकार की शंका करना मना था। इसी प्रकार दूसरा कठोर नियम था भोजन के बारे में। आश्रम के विद्यार्थी बालक निकट के गांवों से भिक्षा में पका-पकाया भोजन ले आया करते थे। वे जो कुछ लाते, गुरुजी के आगे रख देते। फिर गुरुजी सबको भोजन बांटते और उसी में से अपने लिए भी निकाल लेते। रोज का यही नियम था।

आश्रम के अहाते में फलों के बाग थे और दूध के लिए कई गउएं भी थीं।

बालको को दो विषय सिखाये जाते थे—धर्म और युद्ध की कला। जो लड़के बड़े होकर आचार्य या पुरोहित बनना चाहते थे, वे वेद आदि का अध्ययन करते



और धार्मिक संस्कारों, जैसे विवाह, यज्ञोपवीत, यज्ञ, श्राद्ध आदि कराने की विधि और मंत्रोच्चार सीखते थे। जो लड़के सैनिक बनना चाहते थे वे शस्त्र-विद्या और युद्ध के नियम आदि सीखते थे।

आश्रम के गुरु केवल शिक्षक ही नहीं, वे बालकों के माता-पिता भी थे। लड़कों के मां-बाप उन्हें गुरुजी की देख-रेख में छोड़ जाते थे और वे उन्हीं के समान बड़े प्रेम और ममता से अपने विद्यार्थियों की देखभाल किया करते थे।

वे इसका पूरा ध्यान रखते कि उनके मस्तिष्क का ठीक विकास हो, साथ ही उनके चरित्र का निर्माण भी ठीक हो और शरीर भी निरोग रहे। गुरुजी लड़कों की आदतों पर कड़ी दृष्टि रखते। अनुशासन के बारे में तो वे बड़े कठोर थे। कोई बालक बीमार पड़ जाता तो तन-मन से उसकी सेवा करते, लेकिन उससे कोई अपराध हो जाता तो कड़ी से कड़ी सजा भी देते। ऐसा था उनका नियम। उनके आदेशों के बारे में प्रश्न करने का साहस राजा को भी नहीं था।

एक दिन धौम्य ने उपमन्यु को बुलाकर कहा, "बेटा उपमन्यु, राजा एक बहुत बड़ा यज्ञ कर रहे हैं। मेरी इच्छा है कि तुम कुछ दिन उनके पास जाकर रहो। तुम उनकी सहायता करना और देखना कि पंडित लोग किस प्रकार अपना काम करते हैं। उपमन्यु खुशी-खुशी राजा के महल की ओर चल पड़ा। उसे वहां राजा की खास सेवा में नियुक्त



किया गया। उपमन्यु बड़ा ही परिश्रमी और हंसमुख बालक था, इस कारण सभी लोग उससे बहुत प्रसन्न थे। जब कुछ दिनों बाद वह अपने आश्रम में लौटा तो गुरुजी ने उसे बुल भेजा।

गुरुदेव ने पूछा, "बेटा उपमन्यु, क्या तुम इतने दिनों तक उपवास करते रहे?"

"नहीं तो, गुरुदेव," उपमन्यु ने चकित होकर उत्तर दिया।

"मेरी भी यही धारणा थी।" गुरुदेव ने कहा, "तुम काफी हृष्ट-पुष्ट लग रहे हो। तुमने महल के बढ़िया-बढ़िया पकवान छककर तो नहीं खाये?"

"नहीं, गुरुदेव। मैंने महल के पकवान नहीं खाये। सदा की तरह अपना भोजन गांव से ही मांग कर लाता था। गांव महल से तीन मील दूर ही तो है।"

गुरुजी ने कहा, "लेकिन मैंने तो तुम्हें आश्रम में भोजन लाते नहीं देखा।"

उपमन्यु को सहसा याद आया कि आश्रम के नियम के अनुसार उसे सारा खाना गुरुजी के आगे लाकर रख देना चाहिए था। उसने अपना माथा ठोक कर भूल स्वीकार की और गुरुदेव से क्षमा मांगी।

उस दिन उपमन्यु ने भिक्षा में मिला सारा भोजन लाकर गुरुजी के आगे रख दिया। उन्होंने खाना रखवा लिया और इशारे से उसको चले जाने को कह दिया। उपमन्यु सारा दिन प्रतीक्षा करता रहा लेकिन किसी ने उसको खाना नहीं दिया। वह भूखा ही सो गया।

दो दिन बाद धौम्य ने उपमन्यु को फिर बुला भेजा। उन्होंने सोचा कि भूख के



मारे वह अधमरा-सा हो गया होगा। लेकिन उनको देखकर आश्चर्य हुआ कि लड़का पहले की ही तरह चुस्त और स्वस्थ लग रहा है।

धौम्य ने कहा, ''उपमन्यु मैंने दो दिन तक तुम्हारा लाया हुआ सारा भोजन रखवा लिया और तुमको भूखा रखा। मैंने सोचा था कि तुम भूख के मारे कमजोर हो गये होगे। लेकिन तुम तो वैसे ही चुस्त हो और पहले की ही तरह दौड़-भाग कर रहे हो। इसका क्या रहस्य है?''

''गुरुदेव !,'' उपमन्यु ने उत्तर दिया, ''मैं गांव में दोबारा भिक्षा मांगने जाता हूं।''

उसका उत्तर सुनकर धौम्य अप्रसन्न हुए। ''तुमने फिर मेरी आज्ञा का उल्लंघन किया? मैंने कितनी बार बताया है, गांव से दिन में केवल एक बार भोजन लाया जायेगा। बताया था न? उत्तर दो, उपमन्यु!''

''हां, गुरुदेव !,'' उपमन्यु ने क्षीण स्वर में कहा। ''भूख के मारे मैं यह भूल गया था।''

धौम्य ने गंभीर स्वर में कहा, ''तुमको ध्यान रखना चाहिए कि यह गांव केवल हमारे आश्रम की ही सहायता नहीं करता, औरों की भी करता है। उनका कृतज्ञ होने के बजाय तुमने लोभ किया, उनसे दोबारा भोजन मांगा। जाओ, गऊओं को चराने ले जाओ और मैंने जो कुछ कहा है, उस पर विचार करो।''

उपमन्यु का दंड जारी रहा। वह गांव से खाना ला कर आश्रम में दे देता। उसके बाद वह भोजन उसकी आंखों के आगे न आता। आश्रम में सब लोग खाते-पीते, केवल उपमन्यु को खाना न मिलता। इस प्रकार तीन दिन और गुजर गये। गुरुजी इसकी प्रतीक्षा कर रहे थे कि उपमन्यु आकर खाना मांगे। लेकिन वह न आया। यही नहीं, आश्चर्य की बात तो यह थी कि वह उसी प्रकार दौड़-भाग कर अपना काम भी कर रहा था। कमजोरी का नाम-निशान भी नहीं। आखिर गुरुजी ने उसे फिर बुला भेजा और उससे पूछा, ''कहो उपमन्यु! आज



तुम्हें गऊओं के साथ जाने में कोई कठिनाई हुई?"

"नहीं तो गुरुदेव!," उपमन्यु ने कुछ हैरानी से कहा, "जरा भी नहीं।"

बड़े शांत स्वर में धौम्य ने कहा, "मैं इसलिए पूछ रहा था क्योंकि तुमने दो दिन से खाना नहीं खाया है। क्या भूख के मारे तुम्हारी टांगे नहीं कांपती? सिर नहीं चकराता?"

"लेकिन गुरुदेव, मैं भूखा तो नहीं था," उपमन्यु ने कहा।

"क्या?" अब गुरुदेव की बारी थी हैरान होने की। "क्या तुमने इसी अवस्था में भूख पर विजय पा ली है?"

उपमन्यु ने धीरे से कहा, "मैंने गाय का दूध पी लिया था।"

गुरुदेव कुछ देर चुप रहे। उपमन्यु का बुरा हाल था। गुरुजी को क्रोध भी हुआ और दुख भी। कुछ क्षणों बाद वह बोले, "उपमन्यु, मैंने देखा है कि तुम झूठ कभी नहीं बोलते। तुम स्वच्छ रहते हो। पढ़ाई में मन लगाते हो। प्रसन्न रहते हो और दूसरों को भी प्रसन्न रखते हो। लेकिन तुम अपने पेट के इतने वश में हो कि उसकी खातिर नियम तक भूल जाते हो और स्वार्थी बन जाते हो। अपने ऊपर तुम बिल्कुल काबू नहीं रख पाते। और बेटे, मैंने तुम्हें कितनी बार बताया है कि जिस व्यक्ति का शरीर उसके दिमाग का आदेश नहीं मानता, वह कोई भी गलत काम कर सकता है। मेला-तमाशा देखने की खातिर झूठ बोलेगा, भूख को शांत करने के लिए चोरी करने से भी नहीं हिचकिचायेगा, और कीमती वस्त्रों के लिए लोगों को धोखा देगा। क्या यही सब सीखने के लिए तुम्हारे पिताजी ने तुम्हें मेरे हवाले किया था? बोलो, उपमन्यु!"

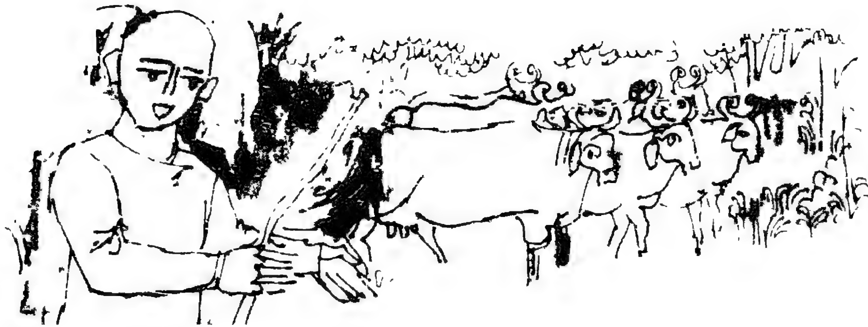
गुरुदेव यदि उपमन्यु को बुरा-भला कहते, डांटते-डपटते तो वह शायद इतना लज्जित न होता जितना उनके ठंडे और दुखभरे स्वर को सुनकर हुआ। उसने उसी क्षण संकल्प कर लिया कि गुरुदेव को कभी अप्रसन्न होने का अवसर नहीं देगा। उसने सिर झुकाकर, विनय से कहा, ''गुरुदेव, जब तक आप स्वयं अपने हाथों से नहीं देंगे, मैं कुछ नहीं खाऊंगा।'' उस दिन उपमन्यु ने कुछ नहीं खाया। दूसरे दिन भी भूखा रहा। तीसरे दिन हमेशा की तरह वह गऊओं को हांक कर जंगल में ले गया तो भूख के मारे उसकी जान निकली जा रही थी। आखिर उसने एक पेड़ के रसभरे पत्ते तोड़कर खा लिये। लेकिन दुर्भाग्य से वह साधारण पेड़ नहीं था। उसकी पत्तियां दवा के काम आती थीं। पत्तियों की गंध इतनी तेज थी कि उपमन्यु की आंखें जलने लगीं, उनमें पानी भर आया और अचानक उसकी दृष्टि जाती रही। वह अंधा हो गया। डर के मारे उसके होश-हवास उड़ गये। टटोलता-टटोलता, गिरता-पड़ता, वह आश्रम की ओर लौटने लगा। वह चाहता था कि अंधेरा होने से पहले आश्रम पहुंच जाय। लेकिन सामने का रास्ता दिखाई नहीं दे रहा था। बार-बार भटक जाता था। वह गलत रास्ते पर जा निकला और एक गहरे अंधेरे गड्ढे में गिर पड़ा। डर के मारे वह पड़ा रोता रहा। धीरे-धीरे रात हो गयी।

जब काफी अंधेरा हो गया और धौम्य ने उपमन्यु को आश्रम में नहीं पाया तो दूसरों से पूछताछ की।

एक छात्र ने कहा, ''वह गऊओं को चराने के लिए जंगल की ओर गया था। गऊएं तो लौट आयीं, लेकिन वह नहीं आया अभी तक।''

गुरुदेव झटपट उठ खड़े हुए और बोले, ''अंधेरा हो रहा है, चलो उसे ढूंढें।''

शिष्यों को साथ लेकर गुरुदेव उपमन्यु को ढूंढ़ने जंगल की ओर चल पड़े।



जहां-जहां वह जाया करता था, सभी जगह उन्होंने देख लिया। उसका पता न चला। अंत में एक अनजाने रास्ते पर चलते-चलते वह जोर-जोर से पुकारने लगे, "उपमन्यु! कहां हो बेटा? मेरी आवाज सुन रहे हो?"

रोते-रोते थककर उपमन्यु सो गया था। गुरुदेव की पुकार कानों में पड़ी तो चौंककर उठ बैठा।

"मैं यहां हूं, गुरुदेव," उसने चिल्लाकर कहा। "इस गड्ढे में पड़ा हूं।"

आखिर गुरुदेव ने उसको देख लिया। वह बेहद डरा हुआ था, उसके कपड़े तार-तार हो गये थे, गालों पर आंसू की लकीरें थीं। गड्ढे के किनारे खड़े धौम्य ने नीचे झांककर उपमन्यु को देखा और पूछा, "तुम इस गड्ढे में क्यों बैठे हो, उपमन्यु?"

अपने दुख और अभिमान को कठिनाई से दबाकर उपमन्यु ने कहा, "गुरुदेव, मैं अपनी इच्छा से यहां नहीं पड़ा हूं। मैं इसमें गिर गया। मैंने भूख के मारे एक पेड़ के पत्ते खा लिये। उनको खाते ही मेरी आंखें जलने लगीं और मेरी दृष्टि एकदम धुंधली हो गयी। मुझे लगता है कि मैं अंधा हो गया हूं, गुरुदेव।" फिर



रुककर उसने पूछा, "गऊएं तो ठीक-ठाक हैं न?"

उपमन्यु के मित्रों ने उसको आश्वासन दिया कि गऊएं सही-सलामत आश्रम में लौट आयी थीं। धौम्य ने उपमन्यु से उस पेड़ का वर्णन करने को कहा जिसकी पत्तियां खाकर वह अपनी आंखें गंवा बैठा था। उपमन्यु बता चुका तो गुरुदेव ने कहा, "उपमन्यु, मेरा विचार है कि तुम यहीं रहो और भगवान से प्रार्थना करो कि वह तुम्हारी दृष्टि लौटा दे। यदि तुम सच्चे मन से प्रार्थना करोगे तो ईश्वर तुम्हारी अवश्य सहायता करेगा। यह तो तुम जानते हो, है न? जब तुम्हारी दृष्टि तुम्हें वापस मिल जाय तो आश्रम में लौट आना।"

धौम्य का फैसला सुनकर उपमन्यु समझ गया कि गुरुदेव उसको चुनौती दे गये हैं। यह उसकी परीक्षा है। उसका मन साहस और गर्व से भर गया। उसे लगा कि वह बच्चा नहीं रहा, अचानक बड़ा हो गया है। उसने विनय से सिर झुकाकर गुरुदेव का आदेश स्वीकार किया। आसन लगाकर ध्यान की मुद्रा में बैठ गया और प्रार्थना में लीन हो गया। उसके मित्र आश्चर्य से मुंह फाड़े उसकी ओर देखते रहे। गुरुदेव ने उनका ध्यान भंग करते हुए कहा, "चलो, आश्रम लौट चलें।" सब लौट गये।



जंगल्ल में बिल्कुल अंधेरा था। डरावनी आवाजें आ रही थीं। लेकिन अब उपमन्यु के मन में जरा भी भय नहीं था। पहले तो वह जोर-जोर से प्रार्थना करता रहा, "हे परमेश्वर, त्रिभुवन के स्वामी, मेरी रक्षा करो। मेरी दृष्टि लौटा दो, प्रभु। तुम दयावान हो, पतितपावन हो, जग के पालनकर्ता हो। मेरी मूर्खता को क्षमा करो, भगवान। मेरी गलतियों को क्षमा करो जिनके कारण मुझको यह दंड मिला।"

फिर उपमन्यु अपनी प्रार्थना में लीन हो गया। उसे किसी चीज की सुध-बुध नहीं रह गयी। उसकी आंखें मुंदी थीं, होंठ हिल रहे थे...।

उसका सारा शरीर शिथिल हो गया था। इतने में उपमन्यु को लगा कि अश्विनी कुमार नामक जुड़वां तारे आकाश से उतरकर उसके सामने आ खड़े हैं। गुरुदेव ने बताया था कि अश्विनी कुमार देवताओं के वैद्य हैं।

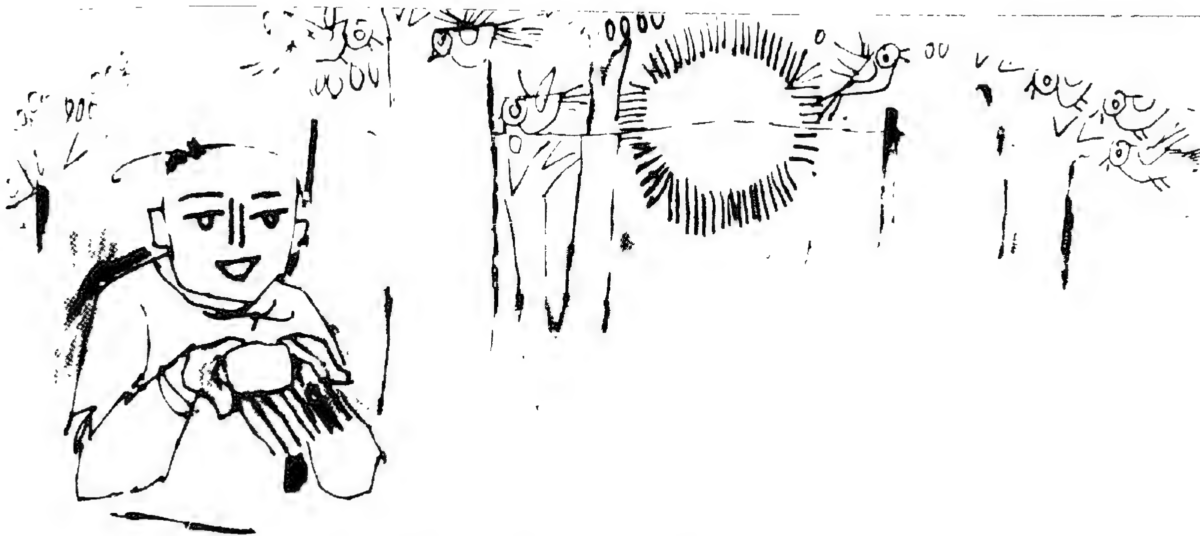
जगमग-जगमग करते वे उसके सामने खड़े पूछ रहे थे, "तुमने हमें पुकारा था, उपमन्यु? हमसे क्या चाहते हो?"

उपमन्यु ने उनसे विनती की कि उसकी आंखें ठीक कर दें। मुस्कराकर अश्विनी कुमारों ने उसको एक टिकिया दी जिसमें दवा मिली हुई थी और उससे खाने को कहा। उपमन्यु एक टुकड़ा तोड़कर मुंह में डालने ही वाला था कि अचानक उसे आश्रम के नियम की याद आ गयी। उसने टिकिया को मुट्ठी में बंद कर लिया। अश्विनी कुमारों से उसने कहा, "मैं इसके लिए आपका कृतज्ञ हूं।"

"तुम इसे खाते क्यों नहीं?" उन्होंने पूछा।

उपमन्यु ने कहा, "मैं इसे अपने गुरु को भेंट करूंगा। हमारे आश्रम का यही नियम है।"

अश्विनी कुमारों ने उसकी ओर चुभती नजर से देखकर पूछा, "तो क्या बीमारी में भी नियम नहीं तोड़े जा सकते?"



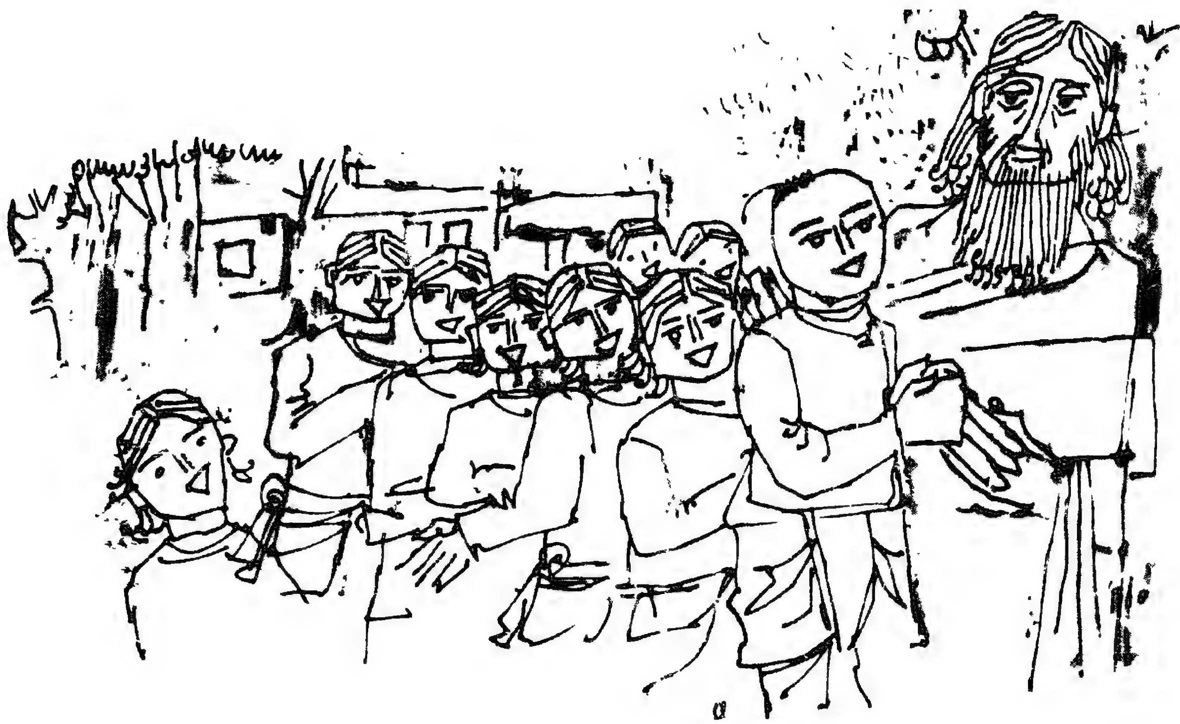
"नहीं, जब तक गुरुजी अनुमति न दे दें," उपमन्यु ने दृढ़ता से कहा।

उपमन्यु को ऐसा लगा कि उसका उत्तर सुनकर अश्विनी कुमारों ने मुस्कराकर एक दूसरे की ओर देखा। फिर उन्होंने हाथ उठाकर उसको आशीर्वाद दिया और उसके बाद आकाश में उड़ गये और फिर तारे बन कर चमकने लगे।

सुबह-सुबह जब उपमन्यु ने आंखें खोलीं तो पेड़ों पर चिड़ियां चहक रही थीं। कैसा चमत्कार! उसकी दृष्टि लौट आयी थी! वह पहले की तरह ही देख सकता था। ऊपर नीले आकाश में रूई के गोले जैसे सफेद बादल तैर रहे थे। ऊंचे-ऊंचे पेड़ों से छन कर सूरज की किरणें कलियों पर पड़ती थीं और वे चटक कर खिल उठती थीं। यह सब कुछ उपमन्यु देख सकता था। उसने वह गड्ढा भी देखा जिसमें वह रात भर पड़ा रहा था। जब वह उठा तो गीली मिट्टी पर पड़ी अपने शरीर की छाप को भी उसने देखा। उसकी मुट्ठी में अब भी कुछ बंद पड़ा था। उपमन्यु ने सोचा, यह जरूर वह टिकिया होगी जो अश्विनी कुमारों ने उसको दी थी।

मुट्ठी में टिकिया संभाले वह किसी प्रकार गड्ढे से बाहर निकला और दौड़ता हुआ आश्रम पहुंचा। इस समय न तो उसको कमजोरी लग रही थी, और न डर। उसके शरीर में स्फूर्ति थी और हृदय आनंद से भरा था।

आश्रम पहुंच कर वह सीधा गुरुदेव के पास पहुंचा। गुरुदेव ने उसे देखा लेकिन कुछ कहा नहीं। वह सुनना चाहते थे कि उपमन्यु को क्या कहना है।



"मैं वापस आ गया, गुरुदेव," उपमन्यु ने कहा। "अश्विनी कुमारों ने मेरी आंखें ठीक कर दी हैं। उन्होंने मुझे कुछ खाने को दिया था।" फिर उसने गर्व से सिर ऊंचा कर के गुरुजी की ओर देखकर कहा, "लेकिन मैंने नहीं खाया। देखिए गुरुदेव, यह चीज अभी तक मेरी मुट्ठी में बंद पड़ी है। जब तक आप नहीं कहेंगे, मैं नहीं खाऊंगा।"

यह कह कर उसने मुट्ठी खोली तो क्या देखता है कि उसमें जंगल की मिट्टी भरी है।

हैरान होकर उपमन्यु ने पूछा, "तो क्या वह स्वप्न था?"

अब धौम्य बोले। उन्होंने कहा, "तुम्हारे लिए तो वह सत्य ही था, उपमन्यु। उतना ही जितना कि तुम्हारी दृष्टि का वापस आ जाना सच है। मुझे आशा है कि तुम्हारी भूख भी लौट आयी होगी, बेटा।"

इसके पहले कि उपमन्यु कुछ उत्तर दे पाता, गुरुदेव ने फिर कहा, "कल हममें से न तो किसी ने खाना खाया और न कोई सोया। चलो, जल्दी से स्नान कर आओ। फिर हम सब चलकर दही-भात खायें।"

भीम और बकासुर

भीम पांच पांडवों में से एक थे। पांडवों को अपने उत्तराधिकार के लिए अपने सौ चचेरे भाइयों से लड़ना पड़ा था। कुरुक्षेत्र के युद्ध के बाद पांडवों को हस्तिनापुर का राज्य मिल गया था। लेकिन उससे पहले उनके चचेरे भाइयों ने, जो कौरव कहलाते थे, उन्हें खत्म करने के अनेकों प्रयत्न किये।

एक बार कौरवों के इस प्रकार के षड्यंत्रों से अपनी रक्षा करते हुए, पांचों भाई अपनी मां कुंती के साथ एकचक्र नामक एक शांतिपूर्ण गांव में पहुंचे। उन्हें वह स्थान पसंद आया और उन्होंने तय किया कि जब तक कौरवों को उनका पता न लग जाय, वे उसी गांव में रहेंगे। वे रहने का स्थान खोज रहे थे कि एक कृपालु ग्रामीण ने उनसे अपने मकान में चलकर रहने को कहा। उसका मकान काफी बड़ा था। पांडवों ने उसको धन्यवाद दिया और उसके घर रहने चले गये।

एक दिन भीम और कुंती बैठे बातें कर रहे थे कि उन्हें लगा जैसे कोई रो रहा



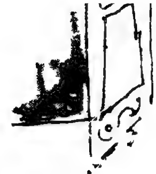
है। कुंती झटपट उठकर देखने के लिए अंदर गयी कि क्या हुआ है। जैसे ही वह कमरे के निकट पहुंचीं, उन्हें ग्रामीण परिवार की बातचीत सुनायी दी।

गृहस्वामी कह रहा था, "अब तो बड़ी देर हो चुकी है। कहीं जाने का समय नहीं रहा। हम सब के भाग्य में यहीं मरना लिखा है क्योंकि हम परिवार के किसी भी व्यक्ति को नहीं छोड़ सकते। मैंने तुमसे कितनी बार कहा कि कहीं ओर चले जायें। लेकिन तुम राजी नहीं हुई। तुमने कहा कि मैं यहीं जन्मी हूं और यहीं मरूंगी। अब तुम्हारी इच्छा पूरी होने वाली है। सिर्फ तुम नहीं मरोगी, हम चारों मरेंगे।"

यह सुनकर हैरानी और भय से कुंती के पैर मानों जम गये।

"इससे अच्छा क्या हो सकता है कि हम दोनों और हमारे बच्चे साथ ही मर जायें। इससे अधिक हमें और क्या चाहिए? लेकिन हमारा दुर्भाग्य, मरना तो सिर्फ एक को है। मैंने बहुत सोचा। यह हो नहीं सकता कि बच्चों में से किसी का बलिदान किया जाय। रह गये आप और मैं। अगर मृत्यु आपको उठा ले गयी तो इन दोनों बच्चों का पालन-पोषण मैं कैसे करूंगी? मैं किस तरह अपना और इनका पेट पालूंगी? कौन-सा काम करूंगी कि इज्जत के साथ निर्वाह हो जाय। बेटी का ब्याह कैसे करूंगी? इस कारण मुझे ही जाना चाहिए जिससे आप रहकर बच्चों की देखभाल करते रहें। फिर यह मेरे लिये उचित दंड भी होगा क्योंकि आपकी इच्छा के खिलाफ यहां बने रहने का हठ मैंने ही किया था। तो अब चर्चा बंद करें। फैसला हो गया।"

कुंती समझ गयीं कि इस नेक ग्रामीण परिवार के ऊपर कोई भयानक संकट आया है। उन्होंने सोचा कि आगे बढ़कर पूछें कि क्या बात है। तभी अचानक लड़की का स्वर सुनकर रुक गयीं। लड़की स्वयं मरने के लिए जाना चाह रही थी ताकि उसके माता-पिता दोनों जीवित रहकर उसके नन्हे भाई का लालन-पालन कर सकें।



अब तो कुंती से नहीं रहा गया। जल्दी से कमरे के अंदर जाकर बोलीं, "क्षमा कीजिए। मैंने आपकी बातचीत सुन ली है। मैं आपके दुख का कारण जानना चाहती हूं। हो सकता है हम आपकी सहायता कर सकें। जो भी संकट है, कम से कम हम सब मिलकर उसका सामना कर सकते हैं।"

कुंती एकदम अंदर आकर बोलने लगीं तो ग्रामीण परिवार चकित होकर उनका मुंह ताकने लगा। उनकी बात सुनकर पुरुष ने कहा, "आप दयालु हैं। आपकी बड़ी कृपा है कि हमारी सहायता करना चाहती हैं। लेकिन हमारी सहायता कोई मनुष्य नहीं कर सकता, कर सकता है तो बस भगवान।"

"मझे सारी बात बताइए," कुंती ने आग्रह किया।

"इस देश का राजा डरपोक है। शासन करना नहीं जानता और मूर्ख भी है। आलसी होने के कारण उसने राजकाज देखने के लिए कई अधिकारियों को नियुक्त किया है जो राजा के नाम से शासन करते हैं। इस गांव का और आसपास के इलाकों का शासक वक नाम का दैत्य है। वह नरभक्षक है — पहाड़ जैसा ऊंचा और भारी-भरकम, लंबे-लंबे उलझे बाल और लाल डरावनी आंखें। जब वह चलता है तो धरती कांपने लगती है। जब वह गरजता है तो आकाश में चिड़ियां डर के मारे तितर-बितर होकर उड़ जाती हैं। हमें प्रतिदिन एक गाड़ी भरकर भात, दो भैंसें और एक आदमी उसके खाने के लिए भेजना पड़ता है। न भेजें तो हमारी खैर नहीं। इसलिए हम सबने फैसला किया था कि प्रति दिन



बारी-बारी एक परिवार से एक व्यक्ति दैत्य का भोजन बनने के लिए भेजा जाय। कल हमारे परिवार की बारी है। हम चार हैं। हम यही तय करने की कोशिश कर रहे हैं कि कल हममें से किसका बलिदान किया जाय।''

इस विचित्र और भयानक कहानी को सुनकर कुंती अवाक् रह गयीं। लेकिन उन्होंने मन में संकल्प कर लिया कि ग्रामीण परिवार की सहायता अवश्य करेंगी। बहुत सोचकर उन्होंने एक फैसला किया।

''अगर आप स्वीकार करें तो मैं एक सुझाव दूं?''

''हम अवश्य आपकी बात मानेंगे। हम लोग तो इतने परेशान हैं कि कुछ सूझता ही नहीं।''

''तो सुनिए,'' कुंती ने कहा। ''आपका एक ही पुत्र है और वह भी अभी बच्चा है। मेरे पांच जवान तगड़े बेटे हैं। उनमें से एक कल भात की गाड़ी और भैंसें लेकर राक्षस के पास चला जाय।''

ग्रामीण ने अपने कानों पर दोनों हाथ रख लिये मानों कुंती ने जो कहा उसे सुनना भी पाप हो।

''देवी, आपकी बात को मैं सुन भी नहीं सकता, उसे मानना तो दूर। अपनी रक्षा करने के लिए आपके पुत्र के बलिदान की बात सोचना भी पाप है।''

कुंती ने बड़ी कठिनाई से ग्रामीण को शांत किया, फिर उसको समझाने लगीं। गर्व से मुस्कराकर बोलीं, ''आप मेरे पुत्रों को नहीं जानते। मैं खास तौर से अपने मंझले पुत्र के बारे में सोच रही थी। मैं भी उसको उतना ही प्यार करती हूं जितना आप अपने बेटे को। लेकिन राक्षसों से लड़ने का उसे खूब अभ्यास है। उसका भी डील-डोल राक्षसों जैसा ही है और चाल तो हवा की तरह तेज है। मेरी बात मानिए, मेरे बेटे को बकासुर के पास जाने दीजिए।''

यह कह कर कुंती अपने कमरे में वापस चली गयीं। उन्होंने अपने पुत्रों को बकासुर के बारे में बताया और कहा कि वह उनमें से एक को राक्षस के पास

भेजने का वायदा कर आयी हैं। भीम ने हंसकर कहा, "मैं जाऊंगा मां। मैं ही इस काम के लिए ठीक हूं। पक्का समझो।"

दूसरे दिन भीम ने बकासुर का भोजन जुटाया और खुशी-खुशी जंगल की ओर चल पड़ा जहां राक्षस रहता था। बकासुर अपने घर से देख रहा था। गाड़ीवान की ऊंचाई, उसके विशाल शरीर, उसकी बलिष्ठ भुजाओं और चौड़ी छाती को देखकर उसने संतोष से सिर हिलाया — "हां, आज खाने में मजा आयेगा।" लेकिन दूसरे ही क्षण उसकी भौंहें तन गयीं। भैंसें कहां हैं?

अगले ही क्षण उसने भीम को जो करते देखा उससे उसका आश्चर्य क्रोध में बदल गया। भीम ने जमीन पर केले का बड़ा-सा पत्ता बिछाया और आराम से बैठ गया। फिर बहुत बड़े बेलचे से गाड़ी में से खाना निकाल-निकाल कर पत्तल पर रखने लगा। राक्षस के देखते-देखते भात, तरकारी और दूसरी चीजें तेजी से भीम के अथाह सुरंग जैसे पेट में पहुंचने लगीं। क्रोध के मारे राक्षस पागल-सा हो गया। बड़े जोरों से गर्जना करता वह आगे बढ़ा और भीम से कुछ ही दूरी पर खड़ा हो गया। भीम ने बक के पेड़ के तनों जैसी टांगों की ओर देखा और फिर पत्तल में भात परोसने लगा। बकासुर और निकट आया।

"कैसे मूर्ख हो तुम जो बक के क्रोध का शिकार बनना चाहते हो। क्या तुम नहीं जानते कि तुम्हें मेरा आहार बनने के लिए भेजा गया है? और मेरी भैंसें कहां हैं?"

भीम ने थोड़ा भात और मसालेदार तरकारी का एक बड़ा ग्रास मुंह में भरा और उसे निगलकर कहा," भैंसें नहीं हैं।"

बक ने गरज कर पूछा, "क्या मतलब?"

भीम ने ठंडे स्वर में समझाया, "गांव में मवेशियों की कमी है। हमें अपनी गऊओं और भैंसों की जरूरत है। गांव के बच्चों को दूध चाहिए न।"

क्रोध से कांपते हुए बकासुर भीम की ओर झपटा। भीम हिला-डुला नहीं।



राक्षस भीम के पीछे खड़ा हो गया और उसको उठाना चाहा। उसने अपनी सारी ताकत लगा दी लेकिन वह पांडव योद्धा टस से मस नहीं हुआ। तब बक ने अपनी दोनों विशाल बाहें उठायीं और भीम की गर्दन पर प्रहार किया।

"अरे, जाओ भी यहां से," भीम ने ऐसे कहा मानों चोट से उसका केवल एक बाल इधर-उधर हो गया हो। "तुम मुझे बेकार तंग कर रहे हो और इस बढ़िया खाने का मजा बिगाड़ रहे हो।"

आश्चर्य और क्रोध के मारे बकासुर जड़ होकर खड़ा रहा। उसकी आंखों के सामने भीम ने गाड़ी का सारा भोजन चट कर दिया, फिर डकार ली और हाथ धोने लगा।

फिर कुश्ती के लिए तैयार होकर भीम ने कहा, "अगर तुम तैयार हो तो आओ दो-दो हाथ हो जायें।"

बकासुर तन कर भीम की ओर लपका। भीम ने बिजली की तरह कूद कर राक्षस को धराशायी कर दिया और उसके पेट पर चढ़ बैठा। बक करवट लेकर भीम के नीचे से निकल गया, और जड़ समेत एक पेड़ को उखाड़ कर भीम की ओर झपटा। भीम ने भी अपने दाहिने हाथ से एक पेड़ उखाड़ लिया और उसको सामने करके बक के वार को रोकने की चेष्टा करने लगा। दोनों योद्धा एक-दूसरे को पेड़ फेंक-फेंक कर मारने लगे। उनकी लड़ाई से घबराकर चिड़ियां डर के मारे इधर-उधर उड़ती रहीं फिर दूर जाकर बैठ गयीं और लड़ाई देखने लगीं। पर्वत

जैसे उन यौद्धाओं की टक्करों से मीलों तक धरती कांपने लगी मानों भूचाल आ गया हो।

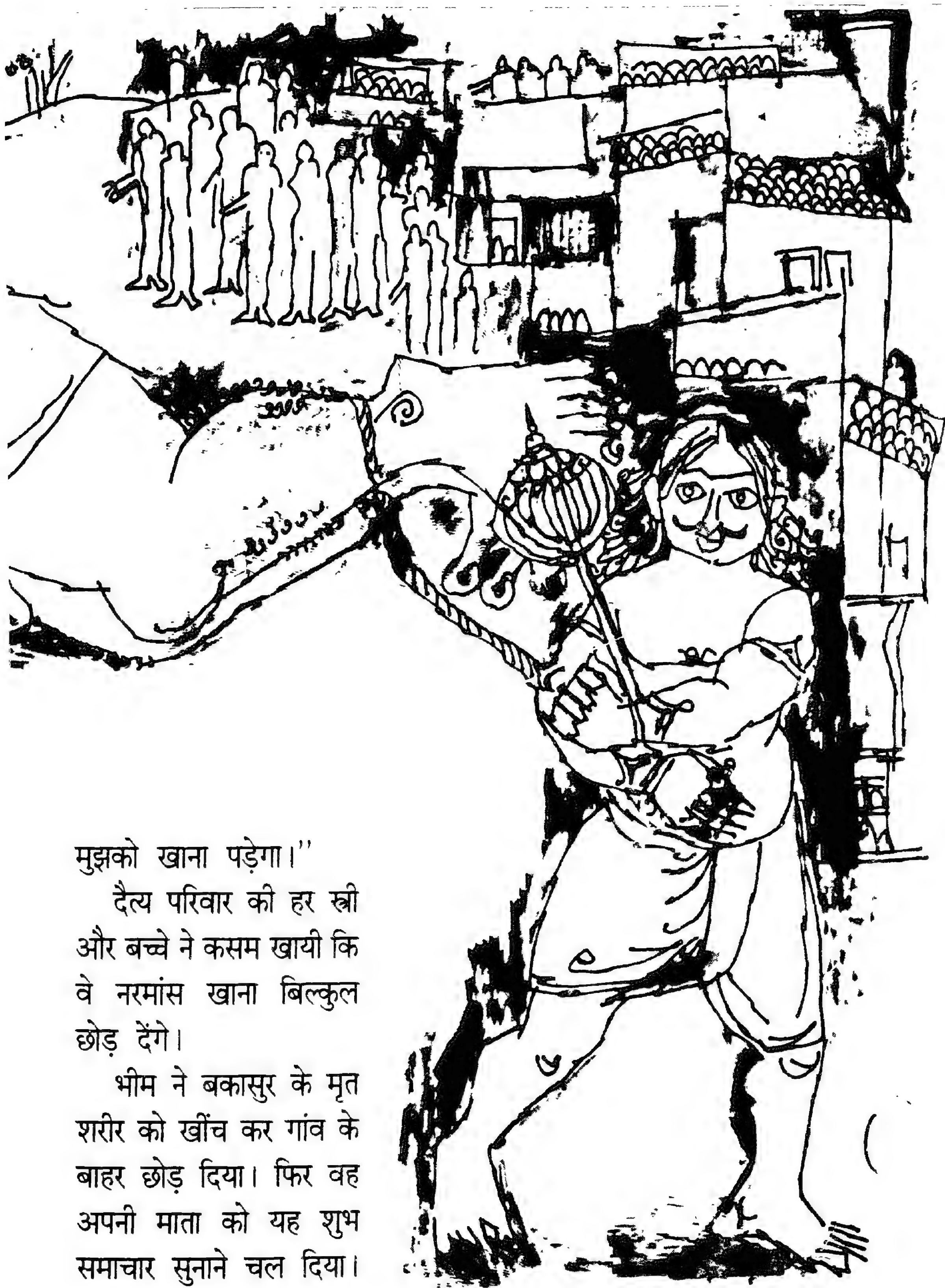
जल्दी ही बकासुर थकान के मारे हांफने लगा। अब वह किसी तरह इस दैत्य से बचकर भाग जाना चाहता था जो उसकी जान लेने के लिए आया था। लेकिन भीम ने उसको जाने नहीं दिया। जब बकासुर भाग जाने की कोशिश करता तो





भीम उसको पीछे खींचकर उस पर लातें और घूंसे बरसाता। अंत में भीम ने इतने जोरों का वार किया कि बकासुर चारों खाने चित्त हो गया। उसकी पीठ पर घुटना रखकर भीम ने उसके सिर और टांगों को इतनी जोर से पीछे मोड़ा कि उसकी रीढ़ की हड्डियां तड़तड़ा कर टूट गयीं। दर्द के मारे बकासुर इतने जोरों से गरजा कि जंगल के शेर और बाघ तक डर के मारे अपनी-अपनी मांदों में जा छिपे। भयंकर गर्जना के साथ बकासुर मर गया।

जब बकासुर के घर वालों और नातेदारों ने मृत्यु से पहले उसकी चीख सुनी तो वे भागे-भागे उस जगह आ पहुंचे और भीम के चरणों पर गिरकर क्षमा-याचना करने लगे। भीम का चेहरा कठोर था, लेकिन उसकी बात न्याय-संगत थी। वह बोला, ''तुम लोग इस जंगल में एक ही शर्त पर रह सकते हो कि आज से तुम नरभक्षक नहीं रहोगे। नरमांस खाना छोड़ दोगे। अगर यह शर्त स्वीकार नहीं तो एकचक्र के किसी भी आदमी को हाथ लगाने से पहले



मुझको खाना पड़ेगा।"

दैत्य परिवार की हर स्त्री और बच्चे ने कसम खायी कि वे नरमांस खाना बिल्कुल छोड़ देंगे।

भीम ने बकासुर के मृत शरीर को खींच कर गांव के बाहर छोड़ दिया। फिर वह अपनी माता को यह शुभ समाचार सुनाने चल दिया।



खांडव वन में आग

बात तब की है जब कुरुक्षेत्र का युद्ध समाप्त हो चुका था और युधिष्ठिर को हस्तिनापुर का राजा बना दिया था। एक दिन कृष्ण और अर्जुन यमुना तट पर बैठे शाम की ठंडी-ठंडी हवा का आनंद ले रहे थे। युद्ध के खत्म होने के बाद, जीवन शांतिपूर्ण हो गया था — यहां तक कि उन्हें अब यह शांति कभी-कभी खलने लगती थी। उन्हें जोखिम और रोमांच पसंद था।

कृष्ण और अर्जुन दोनों बैठे नये मंत्रियों और राज्य के अधिकारियों के बारे में बातचीत कर रहे थे कि उन्होंने देखा कि एक लंबा आदमी लड़खड़ाता हुआ उनकी ओर चला आ रहा है। वह रौबदार लेकिन बीमार लगता था। पास आकर उसने बड़े दयनीय स्वर में कहा, "मुझे भोजन चाहिए। मैं भूख के मारे मरा जा रहा हूं।"

कृष्ण और अर्जुन दोनों ने लगभग एक साथ उत्तर दिया, "हम अभी आपके भोजन की व्यवस्था कर देते हैं । आप जी भर कर खाइए। जब तक भोजन

मंगवाएं कृपा करके बैठ जाइए और आराम कीजिए।" उन दिनों लोग अतिथियों का बहुत सत्कार करते थे।

लेकिन उस व्यक्ति ने सिर हिलाकर कहा, "मुझे साधारण भोजन नहीं चाहिए। कुरकुरा, मजेदार भोजन चाहिए जिसके एक ग्रास से मुंह भर जाय। आपने मुझे नहीं पहचाना? मैं अग्नि हूं।"

अब दोनों ने निकट आकर ध्यान से देखा। सचमुच वह तो अग्नि ही थे, लेकिन इतना बदल गये थे कि पहचान में ही नहीं आये। उन दोनों ने आदरपूर्वक अग्नि को नमस्कार किया, और उन्हें आराम से बिठाकर उनका कुशल-मंगल पूछने लगे। क्या वह बीमार हैं? कौन-सी खास वस्तु खाना चाहते हैं? वस्तु चाहे जितनी दुर्लभ हो उसको पाना चाहे जितना कठिन हो, वह मंगवायी जायेगी और उनके आदेश के अनुसार उसे पकाया जायेगा।



अग्नि ने क्षीण स्वर में कहा, "कुछ पकाने की आवश्यकता नहीं। मेरा खास भोजन तो तैयार है।"

"कहां है? क्या है वह?"

"वह है खांडव वन।"

"आपका मतलब है कि वह खांडव वन में है?"

"नहीं, खांडव वन ही वह वस्तु है।"

कृष्ण ने आश्चर्य से कहा, "तो क्या आप वन को ही खाना चाहते हैं?"

"हां, मैं इसका कारण बताऊंगा," अग्नि ने कहा। "आपने राजा श्वेतकि का नाम सुना है?"

"हां-हां", अर्जुन ने कहा "वही न जिन्होंने बहुत

से दान-पुण्य, यज्ञ और बलि आदि कर के बड़ा यश कमाया है?''

''हां,'' अग्नि ने उदास होकर कहा। ''वह एक दिन अवश्य स्वर्ग जायेगा। लेकिन जब तक वह धरती पर है, एक भी ब्राह्मण उससे वास्ता नहीं रखेगा।''

''आख़िर क्यों?,'' अर्जुन ने पूछा।

''उनके यज्ञ आदि करवाते-करवाते वे लगभग अंधे हो गये हैं। प्रति दिन धुएं के सामने बैठकर सारा दिन कोई वेद-मंत्र पढ़ता रहे तो और क्या होगा, बोलिए? जब देश की सारी लकड़ियों को हवन-कुंड में झोंका जा चुका तो मुझे कच्ची हरी टहनियां खिलायी जाने लगीं। कच्ची लकड़ियों का धुआं कैसा होता है, आप जानते हैं। बस, बेचारे ब्राह्मणों की आंखों और गलों में इतना धुआं भर गया कि वे लगभग अंधे-से हो गये। यहां तक कि राजा को हार कर अपने पड़ोसी राज्यों से ब्राह्मण-पंडित बुलवाने पड़े।''

अर्जुन ने कृष्ण की ओर इस डर से नहीं देखा कि कहीं ऐसा न हो कि आंखें मिलते ही वे दोनों ठठा कर हंस पड़ें। उन्होंने हंसी रोककर पूछा, ''तो क्या यह यज्ञ आदि बहुत समय से चल रहे हैं?''

''हां, बरसों से,'' अग्नि ने उत्तर दिया। ''एक यज्ञ समाप्त होता तो राजा श्वेतकि दूसरा आरंभ कर देते। उन्होंने वह सब बलि चढ़ाया है जो हर क्षत्रिय राजा का धर्म समझा जाता है। फिर एक और यज्ञ किया, जिसके पूरा होने पर हजारों ब्राह्मणों को दान दिया गया। इसके बाद एक और यज्ञ हुआ जिसका उद्देश्य था स्त्रियों और बच्चों का कल्याण।''

कृष्ण ने गंभीरता से कहा, ''यह तो किसी धर्मपरायण राजा के लिए भी कुछ ज्यादा ही है।''

''इससे मेरी दशा क्या हो गयी, देखिए'' अग्नि ने ठंडो सांस भरकर कहा। ''जब मैं आया तो आप मुझे पहचान भी नहीं सके। बरसों से इन यज्ञों में मुझे बाल्टी भर-भरकर सिर्फ देसी घी पिलाया गया है। जरा सोचिए तो, बरसों केवल

घी पीकर रहना कैसा होगा ! मेरा रंग देखिए। वह चमक-दमक, वह आभा कहा गयी, जिसे देखकर स्त्रियों को भी ईर्ष्या होती थी। मैं स्वास्थ्यप्रद, पौष्टिक भोजन के लिए मर रहा हूं। मुझमें बिल्कुल शक्ति नहीं रह गयी है।''

''क्या आप इसीलिए खांडव वन को खाना चाहते हैं ?''

''हां, एक कारण यह भी है। खांडव वन में वह सभी कुछ है जिसके लिए मैं तड़प रहा हूं — सूखे पेड़ जो मुंह में खूब कुरकुरे लगेंगे, हरे रसभरे पौधे, झाड़ियां और लताएं। अपनी अनेक जिह्वाओं में से एक से मैं उनका रस चाटूंगा और जानवर जिनका मांस मेरे होठों को अमृत के सामन स्वादिष्ट लगेगा।''

''आपने कहा था कि यह एक कारण है। तो क्या कोई और कारण भी है ?'' अर्जुन ने अग्नि की बात काटकर पूछा।

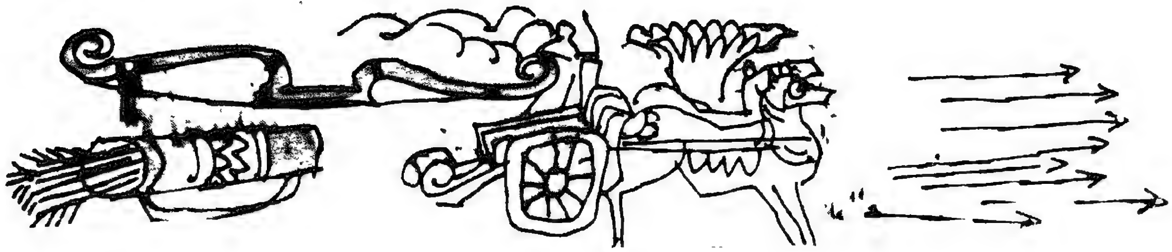
''हां,'' अग्नि को भोजन की बात से बड़ी तृप्ति मिल रही थी। अर्जुन के पूछने पर बड़ी कठिनाई से उस ओर से अपना ध्यान हटाया और बोले, ''खांडव वन स्वर्ग के देवता इंद्र का गढ़ बन गया है। मेरी समझ में नहीं आता कि जब इंद्र के पास इतनी शक्ति है, अनगिनत देवता उसके अधीन हैं, तो उनको अपनी सुरक्षा के बारे में सदा डर क्यों लगा रहता है ?'' धरती और स्वर्ग में वह हमेशा इसी का पता लगाया करते हैं कि कहीं कोई विद्रोही तो नहीं है, या कोई गुप्त रूप से उनकी गद्दी छीनने की कोशिश में तो नहीं लगा है ?'' उन्हें जगह-जगह दुश्मन दिखायी देते हैं।''

''तो क्या इंद्र के मित्र और गुप्तचर खांडव वन में रहते हैं ?''

''और जानते हैं उनका नेता कौन है ? तक्षक।''

क्या ?'' अर्जुन ने आश्चर्य से कहा। ''तो सर्पराज इतने गिर गये हैं कि वे गुप्तचर और भेदिये का काम करने लगे हैं ?''

अग्नि ने कहा, ''वे स्वयं नहीं करते। मेरा उनसे कोई झगड़ा नहीं। उनका रास्ता अलग है और मेरा अलग। लेकिन वन में उनकी जो सर्प सेना है उसमें



उनकी तरह न्याय-बुद्धि तो है नहीं। इसके अलावा वहां पिशाचों, दैत्यों, असुरों और दुष्ट आत्माओं का अजीब जमघट है। वहां मनुष्य-भक्षक हैं, दैत्य-दानव हैं, प्रेत हैं, बैताल हैं, विचित्र पक्षी हैं और भयानक जंतु हैं। जंगल में इस प्रकार के अधम जमा होते रहे, इनकी संख्या बढ़ती गयी तो जंगल का क्षेत्र भी फैलता गया और आसपास के गांव मानों सिकुड़ कर छोटे होते गये। सूर्यास्त के बाद लोग बाहर निकलना नहीं चाहते क्योंकि वे डरते हैं कि जंगली पशु उनके बच्चों को उठा ले जायेंगे। रात भर जंगल से इतनी डरावनी आवाजें आती रहती हैं कि लोग सो नहीं पाते, रात भर डर से पड़े कांपते रहते हैं। कुछ न कुछ करना ही पड़ेगा। जंगल को नष्ट करना होगा।''

अर्जुन ने शंका व्यक्त की, ''क्या आप उस विशाल वन को अकेले ही खत्म कर सकेंगे?''

अग्नि ने चटखारे लेते हुए कहा, ''हां, लेकिन मुझको सहायता चाहिए। मैंने सात बार अकेले उसे चट करने की कोशिश की लेकिन श्रीगणेश भी नहीं कर पाया। स्वर्ग के देवताओं की सहायता से इंद्र स्वयं वन की रक्षा करते हैं। हताश होकर मैं ब्रह्मा के पास गया तो उन्होंने कहा, ''जाओ, कृष्ण और अर्जुन से कहो कि वे तुम्हारी सहायता करें। बोलिए, आप करेंगे मेरी सहायता?''



कृष्ण इस प्रतीक्षा में रहे कि अर्जुन ही निर्णय करें। खांडव वन उनके राज्य में था।




कुछ सोचकर अर्जुन ने कहा, ''हम आपकी सहायता करेंगे। लेकिन हमें शस्त्र चाहिएं। जैसे, मुझे अपने इस धनुष से भारी एक धनुष चाहिएं और बहुत सारे वाण। इस बोझ को उठाने के लिए एक रथ भी चाहिए। रथ को खींचनेवाले घोड़े बिल्कुल सफेद हों। उनकी चाल हवा की तरह तेज हो और जब रथ के पहिए दौड़ें तो मीलों तक बादल उड़ा दें। कृष्ण को भी कुछ हथियारों की आवश्यकता होगी। अगर हमें आप यह सब कुछ दे सकें तो हम खांडव वन को भस्म करने में आपकी सहायता कर सकेंगे।''

अग्नि ने कहा, ''कृष्ण को तो केवल चक्र चाहिए। वह मैं स्वयं उन्हें दे दूंगा। और अर्जुन, आपको जो शस्त्र चाहिएं मैं उन्हें जल-देवता वरुण से दिलवा दूंगा।''

कृष्ण को चक्र मिल गया। वह इस चक्र को इस प्रकार चला सकते थे कि शत्रु का सिर धड़ से अलग करके वह फिर उनके पास लौट आता।

अर्जुन को वरुण से जो धनुष मिला वह कमाल का था। इसके तरकस में तीर खत्म ही नहीं होते थे। एक तीर निकाला जाता तो दूसरा उसकी जगह आ जाता। सृष्टि को बनाने वाले विश्वकर्मा द्वारा बनाया गया एक रथ भी उनको मिला। उसके घोड़े सफेद थे और उसकी साज-सज्जा सुनहरी। वे आकाश में दौड़ते तो लगता कि बिजली कौंध रही है। इसके अलावा वरुण ने अर्जुन को एक बल्लम भी दिया जो फेंके जाने पर बादल की तरह गरजता था।

अर्जुन ने अपना कवच पहना, तलवार कमर में बांधी, हाथों पर नरम चमड़े के दस्ताने चढ़ाये, धनुष उठाया और उसकी कमान खींच कर देखा कि कहीं कोई दोष तो नहीं है। तब हथियारों से भली प्रकार लैस होकर अग्नि से बोले, ''हम तैयार हैं। कृपा कर के जाइए और वन को घेर लीजिए। तब कृष्ण और मैं



अपने-अपने स्थान पर खड़े हो जायेंगे। मैं प्रतिज्ञा करता हूं, खांडव वन अवश्य आपका आहार बनेगा।''

फुंफकारते हुए अग्नि देवता खांडव वन के चारों और घूमने लगे। कृष्ण और अर्जुन आकाश में उठ गये और घने हरे जंगल के ऊपर अपनी-अपनी जगह तैयार खड़े हो गये।

वन में रहने वाले, तरह-तरह के रंग-रूप और आकारवाले जीवों को आग की गंध मिल गयी थी और वे बचाव का रास्ता ढूंढने लगे थे। किसी के भारी सिर और पंजेनुमा पांव थे तो किसी की एक ही आंख थी, और किसी के



कटावदार पंख और लंबे-लंबे नाखून। कुछ तो डर के मारे भागे तो सीधे आग की लपटों में जा घुसे। मशाल की तरह जलते हुए वे आग बुझाने के लिए घास पर लोटने लगे। लेकिन अग्नि की लपलपाती जीभ ने, जो जंगल में फैलती चली जा रही थी, उन्हें अपनी लपेट में ले लिया और वे भयानक आर्तनाद करते हुए मारे गये। विचित्र, भारी काले पक्षी उड़ कर आकाश में बादलों की तरह छा गये, लेकिन कृष्ण और अर्जुन के बाणों ने उन्हें फिर नीचे आग की लपटों में गिरा दिया जहां देखते ही देखते वे जलकर राख हो गये। जंगल के अंदर बहुत से जानवर भय से फैली आंखों के पास आती आग की लपटों को निहार रहे थे। सांपों ने घास मे रेंग कर निकल भागने की कोशिश की, लेकिन अग्नि ने फुर्ती से घास को जला दिया और साथ ही उन्हें भी खत्म कर दिया।

इस अग्निकांड के कारण गर्मी इतनी बढ़ गयी कि जंगल में जो झील और तालाब थे उनका पानी उबलने लगा। मछलियां मरकर पानी पर तैरने लगीं।

जले मांस की दुर्गंध सारे जंगल में फैल गयी। आसपास के गांवों में रहनेवाले लोग शोर, दुर्गंध और उस भयंकर दृश्य से बचने के लिए घर छोड़ कर भाग खड़े हुए।

स्वर्ग के देवता लोग तो कुछ देर तक भयभीत होकर यह कांड देखते रहे, फिर दल बांध कर अपने राजा इंद्र के पास गये। समाचार सुन कर इंद्र के क्रोध का ठिकाना न रहा।

एक देवता ने कहा, "महाराज, आपको शायद स्मरण हो कि तक्षक खांडव में नहीं है। वह अभी कुरुक्षेत्र से वापस नहीं आया। लेकिन अगर वह होता भी तो खांडव को बचाना बहुत कठिन था।"

"क्यों कठिन था?" इंद्र ने पूछा।

"क्योंकि अग्नि अकेला नहीं है। कृष्ण और अर्जुन उनकी सहायता कर रहे हैं। इस समय उनका रथ जलते हुए जंगल के ऊपर खड़ा है।"

यह सुन कर इंद्र क्षण भर चुप रहे। कृष्ण और अर्जुन प्रसिद्ध योद्धा थे और अजेय माने जाते थे। लेकिन देवताओं के राजा इंद्र को इतना अहंकार था कि उन्होंने प्रतिज्ञा की कि खांडव वन की रक्षा भी करेंगे और साथ ही कृष्ण और अर्जुन को परास्त कर सारे विश्व के सबसे बड़े योद्धा कहलाएंगे।

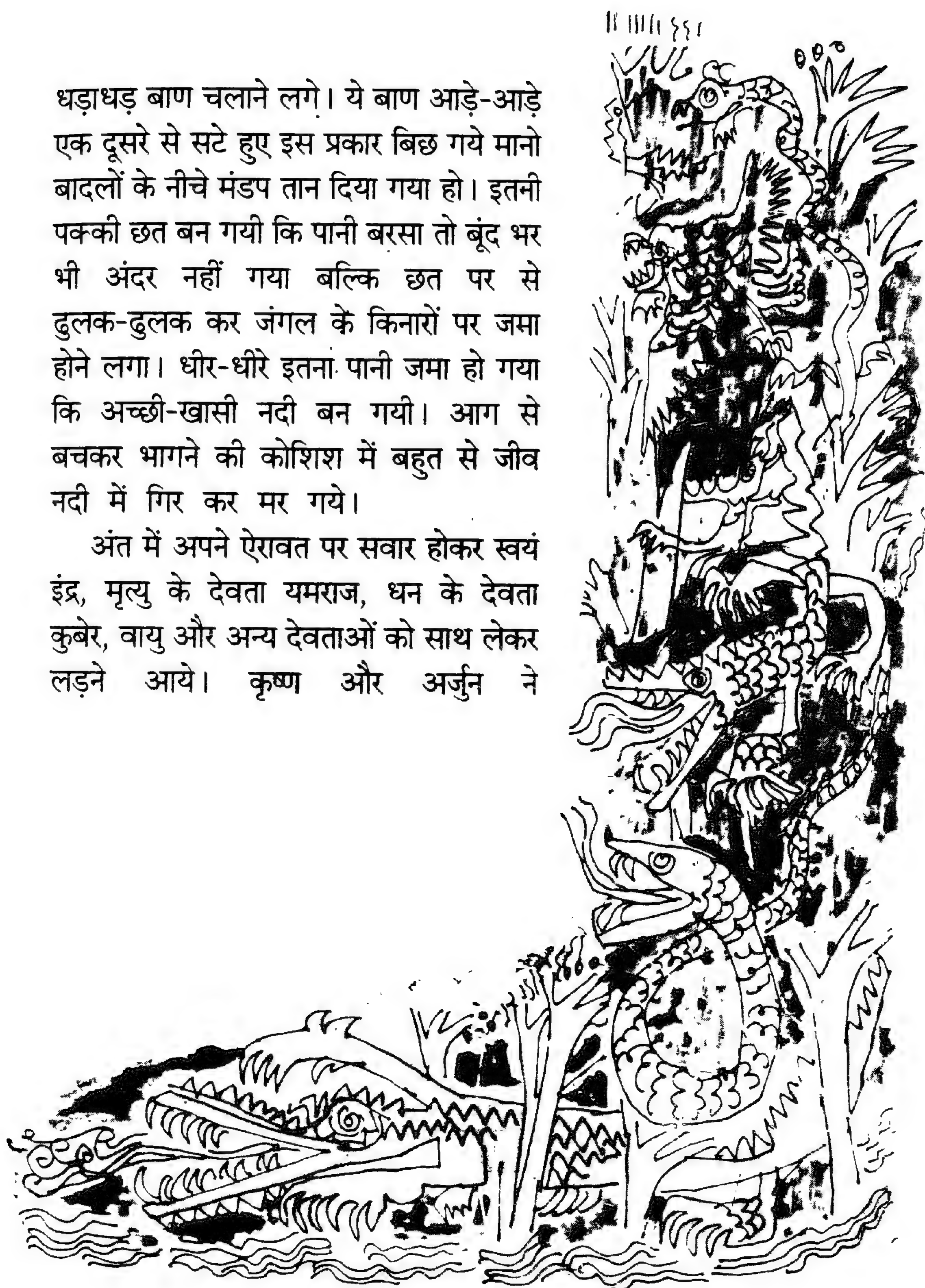
यह निर्णय करने के बाद इंद्र तैयारी में लग गये। उन्होंने देवताओं को आज्ञा दी कि सारे अग्नि वन को मीलों तक गहरे बादलों से ढक दें जो उनका संकेत पाते ही पानी बरसाने लगें।

जब वन के ऊपर बादल जमा हो गये और सूरज ढंक गया तो कृष्ण और अर्जुन वन के ऊपर की ओर, लेकिन बादलों के नीचे से,



धड़ाधड़ बाण चलाने लगे। ये बाण आड़े-आड़े एक दूसरे से सटे हुए इस प्रकार बिछ गये मानो बादलों के नीचे मंडप तान दिया गया हो। इतनी पक्की छत बन गयी कि पानी बरसा तो बूंद भर भी अंदर नहीं गया बल्कि छत पर से ढुलक-ढुलक कर जंगल के किनारों पर जमा होने लगा। धीर-धीरे इतना पानी जमा हो गया कि अच्छी-खासी नदी बन गयी। आग से बचकर भागने की कोशिश में बहुत से जीव नदी में गिर कर मर गये।

अंत में अपने ऐरावत पर सवार होकर स्वयं इंद्र, मृत्यु के देवता यमराज, धन के देवता कुबेर, वायु और अन्य देवताओं को साथ लेकर लड़ने आये। कृष्ण और अर्जुन ने



सबको खदेड़ दिया। यहां तक कि इंद्र के पैर भी उखड़ गये। लेकिन उन्होंने युद्ध से भागकर अपनी रक्षा नहीं की। उन्हें अपना वचन पूरा करना था और किसी प्रकार तक्षक की पत्नी और पुत्र की रक्षा करनी थी। जब इंद्र ने तक्षक से उन तमाम विचित्र जीव-जंतुओं को संभालने को कहा था जिन्हें खांडव वन में भर दिया गया था तो यह वचन दिया था कि उसके स्त्री-पुत्र की रक्षा करेंगे।

जब अपने शानदार वाहन पर इंद्र जंगल के ऊपर आये तो तक्षक की पत्नी ने उन्हें देख लिया और तुरंत लपटों के बीच से उड़कर उनके पास जाने लगी। उसकी सुंदर सुनहरी खाल ताप में झुलस गयी थी। अर्जुन ने उस सुंदर नागिन को





आकाश का ओर आते देखा तो उसे तीर से मार गिराने के लिए निशाना साधने लगे। लेकिन कृष्ण ने उन्हें रोका और नागिन को पुकार कर कहा, ''तक्षक की पत्नी तुम्हारा पुत्र कहां है ?'' मुझे तो वह दिखायी नहीं दे रहा है। क्या उसे जंगल में छोड़े जा रही हो जिससे तुम तो बच जाओ और वह जल कर मर जाय ?''

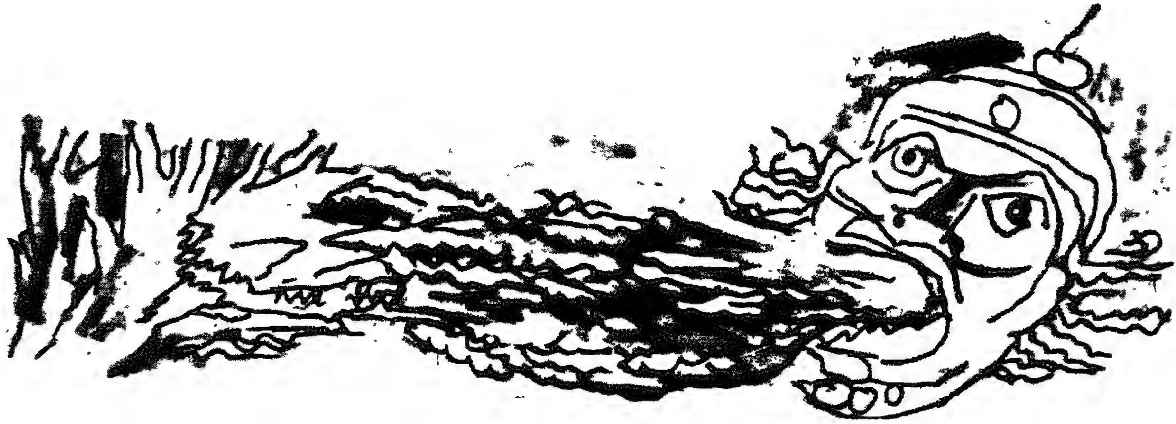


तक्षक की पत्नी ने कहा, ''मेरा पुत्र मेरे पेट में है। मैंने आग से बचाने के लिए उसको निगल लिया। मुझे मारे बिना तुम उसको नहीं पा सकते।''

यह सुनते ही अर्जुन ने अपना धनुष झुका लिया।

खांडव वन पूरे पंद्रह दिन तक जलता रहा। अग्नि ने अपनी अनेक जलती हुई जीभों से जी भर कर मांस, खून, और चर्बी का भक्षण किया। और जब थक गये और तृप्त हो गये तो खाना बंद कर दिया।

चारों ओर मीलों तक फैला और घनी आबादीवाला खांडव वन' अब केवल काली राख का अनंत विस्तार था।



जुपिटर ऑफसेट वर्क्स, दिल्ली-110032 द्वारा मुद्रित

Conserved sequences found in all examples of a particular type of regulatory region in DNA, such as promoters are called consensus sequences. e.g. in a large number of E. Coli promoters the two consensus hexanucleotide sequences are TTGACA and TATATT.

Eukaryotic cells have three different RNA polymerases. One of these makes all of the RNAs that codes for proteins (i.e. the mRNA); the other two make RNA molecules with structural and catalytic roles (such as ribosomal RNAs and tRNAs). All three large multisubunits enzymes that resemble the bacterial enzyme, but the promoters each enzyme recognises are more complex and not as well characterised. It is unclear why both bacterial and eukaryotic RNA polymerases are such complicated molecules with multiple subunits and a total mass of more than 500,000 daltons,

TRANSLATION :

We now begin to look into how single-stranded RNA molecules function during the process of translation, or protein synthesis.

Transfer RNA Molecules Act as Adaptoors that translate Nucleotide Sequences into Protein Sequences.

All cells contain a set of tRNAs, each of which is a small RNA molecule (most have a length of 70 to 90 nucleotides). The tRNAs by binding at one end to a specific codon in the mRNA and at their other end to the amino acid specific by that codon, enable amino acid to live up according to the sequence of nucleotides in the mRNA. Each tRNA is designed to carry only one of the 20 amino acids used for proteen syntesis : a tRNA that carries glycino is designated as tRNA Gly, and so on. Each of the 20 amino acids has at least one type of RNA assigned to it, and most have several. Before an amino acid is incorporated into a proteen chain, it is attached by its carbonyl end to the 3' end of an appropriate tRNA molecule. This attachement serves two purposes. First, it links

Contd...P/4.

cavaleatly the amino acid to a tRNA containing the correct anti codon. Codon - anticodon pairings enable each amino acid to be inserted into a growing protein chain according to the dictates of the sequence of nucleotides in mRNA thereby allowing the genetic code to be used to translate nucleotide sequences into ~~protein~~ protein sequences. The second function of the amino acid tRNA attachment is to activate the amino acid by generating a high energy linkage at its carbonyl end so that it can react with the amino ~~ax~~ group of the next amino acid in the sequence to form a peptide bond. Non-activated amino acids cannot be added directly to a growing polypeptide chain.

Specific enzymes couple Each Amino Acids to its appropriate tRNA Molecule.

Only the tRNA molecule and not its attached amino acid determines where the amino acid is added during protein synthesis. A tRNA finds with its appropriate amino acid out of the twenty different amino acids with the help of the enzyme aminoacyl-tRNA synthetase. There is a different synthetase enzyme for each amino acid (20 synthetase in all).

Initiation Process:

During the initiation phase of protein synthesis, the two subunits of the ribosomes are brought together at the exact spot on the mRNA where the polypeptide chain is to begin. The small subunit is initially loaded with initiation factors (Fig.) and finds the start condon AUG. Then the large subunit is attached. A special tRNA which carries methionine binds to the condon. The metheonine tRNA (formyl methione in prokaryotes) occupies the P-site another aminoacyl tRNA depending on the next condon comes and attaches to the A-site. Amino acids are added to the carbonyl-Terminal end of a growing polypeptide chain. The fundamental reaction of protein synthesis is the formation of a depetide bond between the carboxyl group at the end of a growing polypeptide chain and a free amino group on an amino acid. Throughout the entire process the growing carboxyl end of the polypeptide chain remains activated by its covalent attachment to a tRNA molecule (a peptidyl tRNA molecule).

The Genetic Code:

In the course of synthesis the translation machinery (i.e. ribosome alongwith tRNA) moves in the 5' to 3' direction along the mRNA and the mRNA base sequence is read 3 nucleolides (1 triplet) at a time. Since RNA is constructed of 4 types of nucleotides (adenosine, guanosine, thymidine and uridine), there are 64 possible combinations of three nucleotides (Fig.) (4x4x4x4). Three of these (UAA, UGA and UAG) do not code for amino acids, instead they specify termination of polypeptide chain, they are known as stop signals. Thus there are 61 codon to specify only 20 different amino acids (Fig.).

A ribosome moves stepwise along the mRNA chain:

A ribosome contains three binding sites for RNA molecules, one for mRNA and two for tRNAs. One site called the peptidyl site or P-site, holds the tRNA molecule that is linked to the growing end of polypeptide chain. Another site, called the aminoacyl-tRNA binding site or A-site, holds the incoming tRNA molecule charged with an amino acid. The process of polypeptide chain elongation on a ribosome can be considered as a cycle with three discrete steps. Step 1 - an amino acyl tRNA molecule binds to a vacant A site by forming base pair with a codon in mRNA. Step 2 - The carboxyl end of the polypeptide chain is detached from tRNA and gets linked to the amino end of the amino acyl tRNA at the A site. This reaction is catalysed by peptidyl transference enzyme. Step 3 the new peptidyl tRNA in the A-site is traurtreated to P-site by movement of the rebosome exactly by 3 nucleotides. This is an energy consuming process and requires GTP.

To the free A-site a new aminoacyl tRNA comes at binds and the above processes continue.

Termination:

Three of the 64 codons (UAA, UAG and UGA) in a mRNA molecul are stop codons, which terminate the translation process. Cytoplasmic protein called release factors bind directly to any stop codon that reaches A-site on the ribosome. This binding alters the activity of the enzyme peptidy transferase and causes the release of the newly formed polypeptide, into the cytoplasm. The ribosome then releases the mRNA molecule and dissociates into its two separate subunits, which can assemble again on another mRNA to begin a new round of protein synthesis.

TISSUE CULTURE

Mrs. P. Mishra.

Tissue culture is a general term that is concerned with the study of not only tissues, but also of cells, protoplasts and organs maintained or grown in vitro. In vitro technique (meaning literally in a glass) is relatively a new branch of biological science, is based on the concept of "Totipotency of living cells". a theory put forth by the German botanist Dr. G.Haberlandt in 1902. Totipotency may be defined as the inherent capacity of a living cell to regenerate into a whole organism and is derived from the fact that as each cell of an organism is derived from the fertilised egg it must possess the inherent capacity to divide and give rise to the whole organism. If all of the cells of a given organism are essentially and totipotent, then the cellular differences observed within an organism must arise from the responses of the cells to their microenvironment and to the other cells within the organism. It should be possible to restore suppressed functions by isolating the cells from those organismal influences responsible for their suppression. If there has been a loss of certain functions, so that the cells in the intact organism are no longer totipotent, then isolation would have no effect on restoring the lost activities. The use of culture techniques the scientist to segregate cells, tissues and organs from the parent organism for subsequent study as isolated biological units.

HISTORY:

In 1902 Habulandt was the first to attempt to cultivate isolated plant cells in vitro on an artificial medium .

In the 1930's the subject was put on a scientific foot through the works of Dr. R.J. in France and Dr. P.R. White in U.S.A. In the late 1950's, F.C. Steward and his Co-investigators at Cornell university demonstrated Cellular Totipotency. Their experiment consisted of culturing carrot root cells in a nutrient medium supplemented with coconut milk. The cells developed into plantlets.

Contd...P/2

In 1963 Steward and 1964 Halperin and Wetherell demonstrated the production of thousands of somatic embryos from carrot cells plated on a nutrient medium in petridishes.

Totipotency of cells has now been repeatedly shown in a wide number of plant tissues of diverse origin. This unique property of cells has been imaginatively employed for propogating plants through tissue culture.

Broadly 2 kinds of plant growths are possible.

i) Organised growth: in which organised plant parts continue to grow or when organised structures are formed afresh from unorganised tissues.

ii) Unorganised growth: which is seldom found in nature. This occurs fairly frequently when pieces of plant parts are cultured in vitro. They can be increased in volume by sub-culture and can be maintained in solid or liquid media for long periods.

In practice the following kinds of cultures are most generally recognised

Callus Culture: Cell aggregates arise from disorganised growth of small plant organs or detached plant tissue or previously cultured cells.

Cell Culture: is the Culture of individual cells or populations of cells and cell clumps dispersed in an agitated liquid medium. Also known as Suspension cultures. Here the cells are no longer organised into tissues.

Protoplast culture: is the culture of plant cells that have been isolated without a cell wall.

Organ Culture : is the culture of whole organs of a plant in aseptic conditions. This may be meristem or shoot tip culture, single node culture, another culture embryo culture etc.

Tissue Culture: The maintenance of growth of tissues in vitro, in a way that may allow differentation and preservation of their architecture and/or function.

Contd...P/3

Sub-Culture: Aseptic transfer of a part of a culture to a fresh medium.

Callus Disorganised meristematic or tumor like mass of plant cells formed under invitro conditions.
Explant A fragment of a tissue or organ which is taken from its original site and transfered to an artificial environment for growth or maintenance.
Passage : The duration of each sub culture.

Complete new plants can be derived from tissue culture in 3 ways.

i) from pre-existing shootbuds which are encouraged to grow and proliferate
ii) from shoot morphogenesis when new shoots are induced to grow from unorganised tissues or directly upon explanted tissues of the mother plant.
iii) through the formation of Somatic embryos which resemble the seed embryos of intact plants and which in the same way can grow into seedlings.

Ideally newly formed plants will be genetic carbon copies of parent plants but irregularities to sometimes occur.

The parts to be used as explants depend upon

i) the type of culture to be initiated.
ii) the purpose of the proposed culture
iii) the plant species to be used.

The correct choice of explants and the time of the year at which it is obtained can have an important effect on the success of tissue culture.

Plants growing in the external environment are invariably contaminated with micro-organisms. Such organisms in particular bacteria and fungi compete adversely with plant materials growing in vitro.

Explants must therefore be freed from contaminents before they are transferred to culture and the vessels and media in which cultures are grown must be sterilised and kept in aseptic condition throughout.

Contd....P/4

There are a variety of chemical agents in common use for the surface sterilization of plant material. The choice of agent and the time of treatment depends on the sensitivity of the material to be sterilized. Frequently it is seen that too much of sterilization leads not only to the complete removal of all micro-organisms but is also lethal to the plant tissue. It is therefore, important to determine the optimal conditions for each tissue.

The sterilizing agent should be easily removable, because the retention of such chemicals will seriously affect the establishment of the collus. Repeated washing with distilled water will remove most chemicals. Some sterilizing agents breakdown and become less toxic and the products can be easily washed away. For Ex. Sodium hypochlorite breaks down to give chlorine, the active agent, and sodium hydroxide, which can be removed, while others like Hydrogen peroxide decompose to give harmless components which evaporate.

Following surface sterilization plant materials are transferred to suitable nutrient medium. The composition of the culture medium is an important factor in the successful establishment of a tissue culture. Culture conditions favouring callus growth may not be suitable for organ differentation. Each tissue type requires a different formulation depending on whether the objective is to obtain optimum growth rate or induce organogenesis.

Several media have been developed by various workers to suit particular requirements of a cultured tissue.

A standard or b ' medium consists of a balanced mixture of micronutrient and micronutrient elements (salts of chlorides, nitrates, sulphates, phosphates and iodides of Ca, Mg, K, Na, Fe, Mu, Zu and boron) Vitamins source organic growth factors (aminoacids, Urea and peptones) a source of reduced nitrogen supply and plant hormones.

Contd...P/5

Addition of deproteinized coconut milk, tomato juice, water melon juice, orange juice and other plant extracts, YE, ME, or protein hydrolysate brings about mitosis in quiescent cells and has proved beneficial though not essential in cases where the tissues do not divide easily on a purely synthetic medium. Different nutrient media have been formulated by various workers-

Iron is added in the form of ferric citrate or as FeEDTA (ferric sodium - ethylene diamime-tetra acetate) in order to ensure its availability over a wide range of PH of the medium.

The PH of the nutrient solution which is a balanced mixture of macro and microelements is adjusted to 5.6-6.0 by the addition of 0.1 HCL or NaoH.

The growth regulator requirments for most Callus cultures are auxin and cytokinin. Auxins, a class of compounds that stimulate shoot cell elougation, resemble IAA in their spectrum of activity. Cytokinins, which promote cell division in plant tissue regulate growth and development in the same manner as kineti (6- furfuryl amino purine) cytokinins are mainly N^6- substituted aminopurine derivatives. Auxin-cytokimin supplements are instrumented in the regulation of cell division, cell elougation, cell differentation and organ formation. Gibberellins are rarely added to culture media, altho GA_3 has been used in apical meristem cultures (Morel and Muller 1964).

The auxins most frequently employed are IAA, NAA (α-naphthalemacetic acid) and 2,4-D(2,4-dichlorophenoxyacetic acid). IBA (Indole-3 butyric acid) is a particularly effective rooting agent. IAA is a naturally occuring auxin, but unfortunately it is readily degraded by light and enzymatic oxidation. Because IAA oxidase may be present in cultured tissues, IAA is added to media in relatively high concentrations (1-30mg/l). The most effective auxin for callus proliferation for most cu... cultures is 2.4-D. The most widely used cytokimins in culture media are kinetin, benzyladenine and Zeatin. Kenetin and benzyladenineare synthetic compounds, whereas Zeatin occurs naturally.

Contd...P/6

Culture Techniques

The first techniques developed for callus culture were simple and employed media solidified wilt agar, gelatin or silica gel. The q great merit of this form of culture is its extreme simplicity. Only simple standard laboratory glassware is required and there is no need for complex mechanical devices or elaborate containers. In addition large numbers of cultures can be accomodated in a small space. However, a survey of the literature on plant tissue cultures will show that solid media have now been largely relegated to the establishment and maintenance of callus cultures. Much of the recent critical work on nutrition metabolism and growth has been performed with liquid media. The reason for this are the limitations one comes across when culturing on a solid media. Firstly, only one part of the callus or explant is in contact with the surface of the medium. It is likely, therefore, that as culture proceeds, inequalities in the growth response will arise in response to the nutrient gradients set up between callus and medium. Similarly there may be gradients in the exchange of respiratory gases due to occlusion of the base of the explant. Gradients of toxic waste products may be establish ed. A further disadvantage observed in that cultures grown on solid media cannot be transferred to liquid media without some disturbance to the tissue. Despite these limitations the culture of callus on solid media with agar remains the method par excellence for the routine maintenance of cultures and is still used for experimental investigations.

The culture of tissues in unshaken liquid media has all the advantages of the solid medium methods and many of the disadvantages are absent. Here the tissue is placed on an ash-less filter paper sppp support held at the interface of the medium wilt the air in the test tube. The filter paper acts as a wick providing nutrient while keeping the tissue in the gas phase.

The culture of explants agitated in a liquid medium eliminates many of the disadvantages ascribed to the culture of tissues in stationary culture. Movement of the tissue in relation to the nutrient medium facilitates gaseous exchange, removes polarization of the tissue due to gravity, and eliminates nutrients gradients within the medium and at the surface of the tissue. These can be placed under two categori

1) Continuous immersion: techniques in which the tissue is always in contact with the culture medium and the mixture is shaken or stirred continuously.

2) Periodic immersion: Techniques in which the tissue spends periods immersed in the liquid medium alternating with period in air. Such an arrangement ensures adequate mixing as well as providing efficient gaseous exchange.

Callus cultures require to be transferred periodically to a fresh nutrient medium. Extensive growth leads to the exhaustion of nutrients, drying out of solid media or concentration by evaporation of liquid media and the accumulation of tissue metabolism. Cultures maintained on agar at 25°C or above require to be sub-cultured every 4 to 6 weeks. In the early stages of callus development it may be convenient to transfer the whole piece of tissue but the sub-culture of established callus demands the frequent ~~such~~ sub-division and transfer of separated pieces. In this case it is important to transfer small healthy looking pieces to the surface of fresh agar medium. Failure to transfer cultures ultimately leads to the death of the callus ~~which~~ while a sub-culture from necrotic callus tends to grow much less actively than one taken from an actively growing healthy culture.

The general growth characteristics of a callus involve a complex relationship between the plant material used to initiate the callus, the composition of the medium and the environmental conditions during the incubation period. Establishment of a callus from the explant can be divided roughly into 3 developmental stages (i) induction (ii) cell division and iii) differentiation. During the initial induction phase metabolism is stimulated as the cells prepare for division. The length of this phase depends mainly on the physiological status of the explant cells as well as the cultural conditions. Subsequently, there is a phase of active cell division as the explant cells revert to a meristematic or "dedifferentiated" state. The third phase involves the appearance of cellular differentiation and the expression of certain metabolic pathways.

A homogeneous callus consisting entirely of parenchyma cell is rarely formed. Cytodiffrentiation occurs in the form of ~~traxxx~~ tracheary elements, sieve elements, suberised cells,

secretory cells and trichomus. Small nests of dividing cell form "meristemoid" or vascular nodule that may become cent for the formation of shoot apices, root primordia or recip embryos. One serious problem associated with the use of ca cultures, as well as other cell culture systems, is genet instability resulting in variations in phenotypes within t cell population. Phenotypic variations arising during cult may have either a developmental (epigenetic) or a genetic basis. Genetic variations may involve chromosome aberrat nuclear fragmentation and endo duplication resulting in polyploidy. The frequency of these nuclear abnormal usually increases with the age of the culture, and the cul ral conditions may act in a selective manner. Certain ancu or polyploid cells might gain an advantage in division rat over the normal cells Proliferate to a greater extent, and ultimately remove the dominant cell line of the culture.

The first indication that in vitro organogenesis coul be chemically regulated to some extent was given by Skoog (1944). He found that the addition of auxin to the medium served to stimulate root formation, whereas shoot initiati was inhibited. Subsequently it was found that adenine sulph was active in promoting shoot initiation and this chemical reversed the inhibitory effect of auxin. The studies of skoo and his clleagues led to the hypothesis of that organogene is controlled by a balance between cytokinis and Auxin. A relatively high auxin:cytokinis ratio induced root formati in tobacco callus, whereas a low ratio of the same hormone favoured shoot. The formation of floral buds, vegetative buds, and roots has been demonstrated in thin cell-layer explants of several species by regulating the auxin:cytoki ratio, carbohydrate supply and environmental conditions.

Torrey(1966) - Organogenesis in callus starts with the formations of clusters of meristematic cells (meristermoids capable of responding to factors within the system to prod a primordium. Depending on the nature of the internal facto the stimuli can initiate either a root, a shoot or an embryoid. Many observations on organ formation in cultured tissues support the hypothesis that localised meristematic activity precedes the organised development of roots and shoots. The factors that regulate the origin of these meristematic zones are not understood. Since these zones ar located in the vicinity of the tissue-medium interface, it

has been suggested that physiological gradients of substances diffusing from the medium into the tissue may play a role in determining the loci at which meristemoids are formed.

Once the cultures are established these are sub-cultured for the increase in volume. The duration of each subculture is called a passage. Sub-culture is usually done at interval of 4-6 weeks, length of which depends upon the rate of growth.

Plant propagation through tissue culture also termed as micropropagation which is generally divided into four stages:

Stage O - mother plant selection and preparation
Stage I - establishing an aseptic culture
State II- Production of suitable propagular
Stage III- Preparation for growth in the natural environment.

Micropropagation is usually very much more rapid than the traditional methods. It cannot only provide increased rate of propagation but can facilitate vegetative nultiplication of plants that had previously been proved difficult or impossible to propagate. The efficiency and reliability of vegetative propagation through micropropagation is important for a number of reasons.

In the past, agronomists tailored the environment to suit the crop but plant breeders have to tailor the crop to suit the environment. Much has been and continues to be accomplished by the use of conventional, non-molecular tool of genetics. We have yet to exhaust the potential of classical genetics to improve crop productivity. But with increasing pressure for further improvements in Crop plants, crossability barrier, linkages, variability in gene expression, low selection efficiency for complex traits etc., pose limitations of conventional breeding techniques effective use in the future. The new tools of genetics which include tissue culture techniques offer new possibilities in solving some of these problems.

Meristem, Shoot-Tip and Bud culture:

These techniques are alternative means of asexual propagation of economically important plants. The explant of meristem culture may either be the apical dome

(apical meristem) or more frequently, the apical dome plus a few sub-adjacent leaf primordia. The cultures are initiated from either terminal or axillary buds usually with the stem segment attached, using either a growing or dormant shoot. Plants derived from meristem, shoot tip and bud cultures are generally phenotypically homogenous them indicating genetic stability. Application of these tissue culture techniques for rapid cloral propagation is highly desirable in cases such as (a) problem sps. - do not produce seeds or produce very few seeds, produce viable seeds, have slow rate of multiplication. When advanced breeding lines have been identified it is important to propagate as many seeds or plants as is possible to permit variety release. At this point tissue culture may be used in conjuction with other asexual methods to propgate clonal lines for seed production. Such an approach was used in Equador to establish elite clones of Pyrethrum(Chrysathemum cinerariaefolium's) for commercial production. In some cases cultivated crops, particularly eash crops, where each individual plant is valuable, may be cloned using tissue culture.

Plants obtained through conventional vegetative propagation are liable to accumulate systematic viral, bacterial or fungal infections, disease free individuals obtainable only in small numbers will similarly need rapid multiplication.

It has been observed in a large number of plant species that the concentration of infective viruses is low in the apical meristem of a plant. The lack of vascular differentiation in the meristem impaires intercellular movement of viruses and the active metabolism of mitotic cells precludes viral infection. In certain crops or ornamental species, it has been economically useful to develop in vitro techniques to produce virus - eradicated plants. These species include the elimination of mottle virus from straw beery, potato virus x from potato, couliflowers mosaic virus from cauliflowers.

Plants regenerated from callus may also be free of viral infection. Callus tissue is similar to meristematic tissue as cell mutosis is rapid and vascular differentiation is incomplete. Virus-free plants have been regenerated from callus in tobacco, geramium, Gladiolus and potatoes. Although callus tissue results in virus-free plants, chromosome abnormalities have been documented in plants regenerated from callus tissue.

Anther Culture:

The advantage of the in vitro production of haploids over the conventional method is the shortenning of time to achieve homozygosity. Since homozygosity is attained in one step, one to 5 years of selfing and reselection which is done in curcumtienal breeding could be bypassed. Since no further segregation occurs each plant derived from anther culture is a potential new variety and could be screened immediately for desirable characters. Korea was able to release an anther cultured derived variety (Hwas embryo) in 5 years, in contrast to ten or more years required to develop a variety through conventional means. Rubber trees from selected pollen plants with increased yields of latex, superior growth vigor, early bloom, and increased cold resistance were also reported. Likewise, winter bread wheat 'Florin' has been released as a commercial winter wheat variety. It is the first wheat variety developed by anther culture method in the Western World.

Anther culture also allows faster production and selection of useful mutants. Since haploids possess only are set of alles at each lacus, it is possible for recessive mutants to be detected. In this way, difficulties in the selection of mutants due to dominance are negligible and most, if not all genes, are expressed. This allows easy isolation of recessive mutations because they are expressed immediately. Although in anther culture as in mutation breeding it is not difficult to generate variants and most will be deleterious by virtue of some metabolic imbalance which may be expressed as reduced vigour or fertility, rice, plants with larger seeds, higher levels of seed protein, shorter statue and more highly tilled than the cultivar were obtained from selfed anther-derived plants.

Cell culture:

Many secondary plant products such as dyes, fragrances and drugs are produced by the plants often in very minute

quantities as defense mechanism for their survival.

In vitro culture of different explants could be helpful in isolating and increasing the yield of sp. plant cells which produce such compounds. Moreover, in vitro culture yield products which are more easily purified. The production of secondary plant products has been achieved by especially by selecting high-producing cell lines either by visual selection as in the case of pigmented products or by chemical analysis of the cells as in the case of colourless compounds.

Shikonim , a red naphthoquinone pigment used for medicine, dye and cosmetics is isolated from the purple root of Lithospermum erythrorhizor, a perennial herb native to Japan, Korea and China. Japnese scientists have devised a cell culture system in which the cells containing the red pigment can grow faster and yield higher compared with the whole plant. Other important secondary plant products are Vinblastine and Vincristine which are isolated from Catharanthus roseus. Berberine are important pharmaceutical alkaloid that has antibacterial and anti-inflammatory activities (12.7%) from the roots of coptis japanica (2.4%) more than 5 years). Yield of digitoxin which is the most active principle of Digitatis, a powerful cardiac stimulant and directive, can be increased by as much as 400% in tissue cultured with GA3.

Cell cultures which could be induced to form plants through somatic embryogenesis have several advantages. This is extremely important in the exploitation of hybrid vigour in crops. FI hybrids may yield more than either parent-better known as hybrid vigour. The constraint in the use of hybrid technology is the high price and limited supply of hybrid seeds. An alternative is the mass production of encapsulated somatic embryos from FI seeds.

Somacloral variation which refers to the genetic variability observed in plants, and their progeny from tissue and cell cultures could be utilised in increasing crop productivity. Somacloral variation has been observed in economically important plant sps. which include tomato, maize, potato aljalja tobacco, rice etc.

Plants regenerated from protoplasts of the potato variety Russelt Burbank were not precise carbon copies of the parent. Sheposd(1982) examined over 10,000 plants regenerated from protoplasts and demonstrated that each differed in some way from the parent. The protoclomes showed variation in tuber colours, weight, specific gravity length, width and height, maturity date and photoperiod requirements. A few were more resistant to Alternania Solari toxin than the parent. and some should field resistance to early blight About 2.5% of protoclones screened were resistance to phytophthora infestans.

Embryo Resource

Interspecific hybridization is an important tool for introducing valuable trails from wild species into the gene pools of cultivated plant species. Usually, such crosses do not yield any agriculturally beneficial hybrids primarily because in the transfer of beneficial alien genes across interspecific or intergeneric barriers, too many deterious genes are also introduced. The resulting disturbance in equilibrium between the growth processes of the maternal tissues, embryo and endosperm lead to embryo mortality and seed collapse. These post zygotic in compatibilities can be overcome by embryo culture or embryo rescue.

Embryo rescue has been useful in the hybridization between two jutespecies Corchorus olitorcinus and C. capisularis. Here improved quality with resiatance to pest and diseases were incorporated on one genotype. Wide hybridization is now being utilised in rice. Genes for brown planthopper resiatance have been transferred from the wild sps. O. officinialis into cultivated rice by Embryo rescue.

Synthetic seeds:

Somatic embryo are of typical bipolar nature and resemble the zygotic embryos found in the seed. Now-a-days, biotechnologists are working on the possibilities of producing artificial or synthetic seeds from somatic embryos of important agricultural and forest plants. In "Synthetic seed", a somatic embryo is generally encapsulated in some nutrient gel which acta as the endosperm for the somatic embryo. These synthetic seeds can be stored and transferred easily and can be sown in the field beds directly.

Protoplast Isolation and Fusion:

Somatic hybridization which can be accomplished by protoplast fusion is a means of overcoming prezygotic incompatibilities in interspecific, intergeneic, and inter-familial crosses. This technique is also important in the transfer of cytoplasmic traits such as male sterility and herbicide tolerance.

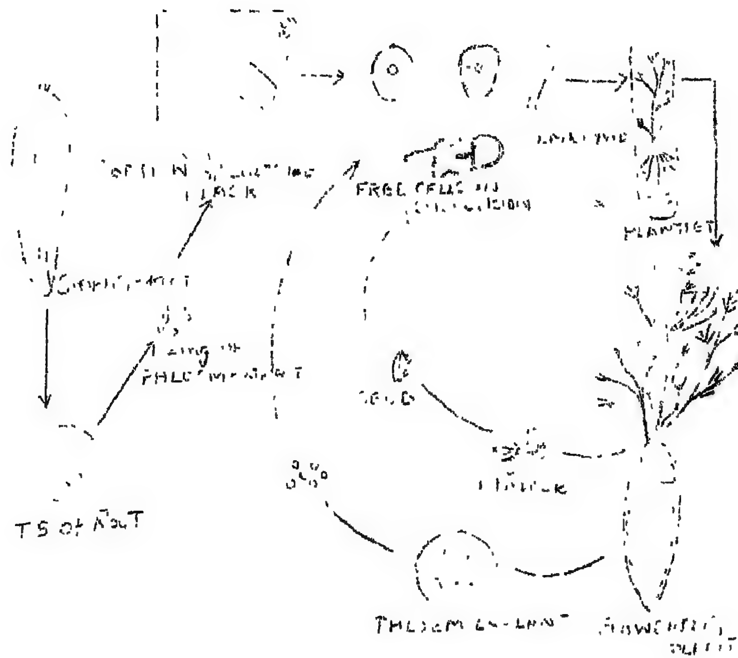
Protoplast fusion is made possible by the use of poly-ethylene glycol or by electro-fusion. Fused protoplast when properly selected and regenerated into plants may exhibit combined characteristics of both . There are some successful somatic hybrids obtained from protoplast fusions in Brassica, Nicotiana and Solanum. Studies in the transfer of resistance to bacterial wild fire diseases and fungal shank from Nicotiana rustica into Nicotiana tabacum were attempted usin somatic hybridization (Patnak et al 1982).

Recombinant DNA Transfer

This technique involves the identification of the desired genes, isolation, duplication and insertion into a receipient cell. The goal is not only to insert the gene, but to have the gene expressed.

The transfer of genes should be done by vectors which ca the desirable gene. The most promising vectors so far seems to be the tumour inducing(Ti) plasmid carried by Agrobacterium tumefaciens. This bacterium causes tumour growths around the root crowns of plants. It infects only dicotyledonous plants and its virulence is due to the Ti plasmid, which when it is transferred to plant cells induce tumours. Once xx inside the cell, a smaller segment of the Ti plasmid, called T-DNA, is actually incorporated into the chromosomes.

Since A. tumefaciens does not infect important cereals such as maize, rice etc. other gene transfer techniques that are potentially applicable to all sps. are being developed. One of these techniques is electroporation which uses brief, high voltage electric shocks of few microseconds in xx duration, which opens pores in the membranes of plants protoplasts thereby facilitating the introduction of plasmids carrying desirable genes.



PLANT CELLS ARE TOTIPOTENT

PROSPECT OF PLANT PROTOPLAST TECHNIQUE FOR GENETIC MANIPULATION

S. N. Patnaik
P. G. Deptt. of Botany
Utkal University
Bhubaneswar-751004

Plant cell differs from animal cell by possessing a cell wall which is made up of cellulose. When the cell wall is removed, the wall less cell is called as protoplast. This protoplast is perceived when plasmolysis occurs in a plant cell. Ever since the protoplasts were recognised, attempts had been made to isolate them from plant tissues and induce fusion in them. Klerker (1892) was successful in cutting the cell wall in leaf tissue plasmolysed in 0.4 M potassium nitrate and through controlled deplasmolysis he was able to release the protoplasts into the medium. Kuster (1910) was the first to achieve fusion between sub-protoplasts as well as the freely isolated plant protoplasts. Michel (1937) standardised the technique and used light microscopic markers to induce anto-plastic (between sub-protoplasts), homoplastic (protoplasts of the same species) and heteroplastic (protoplasts of different species or geneva) fusions. Hofmeister (1954) induced fusion in plant protoplasts by suspending them in isotonic solution such as sea water. However, none of these methods was efficient or reproducible.

The discovery of the chance fusion of two mouse tumor cell lines grown together in the same culture plate by Barski et al. (1960) opened up the prospect of animal cell fusion even in remote cell lines like mouse and man. Such hybrid cells were not mere curiosities since there were preferential loss of chromosomes of different parental lines in the hybrid cells. It came as a handy tool for genetic analysis and gene mapping in higher animals including man.

Almost simultaneously in the 1960's. enzymatic methods for large scale production of protoplasts in plants were discovered.

Contd...P/2

Take-be et al. (1968) developed the procedure of sequential engyme application by treating plant tissue with pectinase for maceration and celulase for cell wall degradation respectively. On the other hand, power and cocking (1968 introduced mixed engyme approach in which plant cells were plasmolysed in the presence of mixtures of pectinase and cellulase. In such procedures, when isolated, protoplasts some times undergo spontaneous fusion. However, in mixed engyme method, fusion within the sympla m became more frequent due to the plasmodesmatal connections. The frequency of such undesirable spontaneous fusion was prevented either following sequential engyme procedure or plasmolysing the cells prior to mixed engyme treatment there by severing the plasmodesmatal connections. Though the spontaneous fusion bodies arising from such homokayotic fusion were of no significance the observation that cell wall is automatically formed around such fused protoplasts and the two nuclie normally fuse and divide in them, generated hope for fusing protoplasts of different species of plants. With the success of culturing protoplasts, raising callus from them and regenerating whole plants from such calli opened a new method of plant hybridization through the fusion of somatic cells. Since the incompatibility barrier, such as the prevention of key steps in pollination, pollen tube development, fertilization and embrgo or endosperm development encountered in sexual hybridization between remote species was found to be nonexistent in somatic hybridization, success could be achieved soon not only in fusing cells derived from taxonomically distinct plants but but even bringing about inter-kingdom fusion between plant and animal cells.

The success of somatic hybridigation depended upon the efficient fusion between protoplasts grown in culture and soon fusion methods were standardsed by employing different fusogenic agents. Power et al. (1970) using sodium nitrate brought about fusion of protoplasts which was successfully employed in many protoplast systems. Keller and Melchers (1973) used mannitol solutions containing calcium ions buffered at high PH (10.5) and this method is

Contd...P/3

now found to be very efficient in widely diverse kinds of protoplast systems. Kao and Michayluk (1974) assessed the ability of polyethylene glycol (PEG) which was though found to have moderate levels of fusion could be applied to a wide range of plant and animal organisms. PEG and Ca^{+2}/high pH could also be used together and even in a veyy "small scale fusion", higher efficiency was recorded for both the agents (Patnaik, 1987). In addition to these three agents, several chemical compounds have now been tested and as many as 20 compounds are now identified which promote cell hybridigation with nearly same efficiency as PEG (Klebe and Mancuso, 1981).

As alternate to chemically induced protoplast fusion Zimmermann and Scheurich (1981) introduced a technique of high frequency fusion of plant protoplasts by electric fields. This was described as a two step process. First, the membranes of neighbouring protoplasts are brought into contact with a nonuniform AC field in a process called dielectrophoresis. Protoplasts, thus in transient dipoles aggregate between the electrode in the form of "Pearl Chains". The points of contact between the plasma membranes of the neighbouring protoplasts are broken down electrically with a short DC pulse. Membrane reorganisation following the DC pulse results in cell fusion.

Though the exact mechanism of membrane fusion is still debatable, some suggestions have been advanced mostly on the basis of chemically induced fusion. Ahkong et al.(1975) held the view that exogenous chemical agents could induce perturbation of the membrane bilayer with increased fluidity of the lipid region which would allow aggregation of inter-membraneous protein particles at places. When the membranes are closely opposed at regions where proteins are now absent, intermixing of the disturbed lipid molecules results in the coalescence of the adjacent bilayers. As regards the role of chemical furogens described, they are believed to play the role of establishing intimate contact between neighbouring membranes, alter surface negative charge of the membranes, trigger changes in membrane structure and permeability resulting fusion.

Contd...P/4.

With the development of technique of enzymatic isolation of protoplasts, their culture and regeneration of whole plants, a new tool could be available for genetic manipulation. While protoplasts as such could be amenable for the entry of exogenous genetic materials and even cell organelles resulting in genetic transformation, fusion of protoplasts opened a new vista for bringing together widely diverse kinds of genomes otherwise not possible through sexual breeding. The aspect of somatic hybridigation may be many fold : (i) synthesis of amphidiploids between sexually incompatible species, (ii) producing heterozygous lines within the same species which ordinarily reproduce by vegetative means, (iii) transfer of part of a nuclear genome to another through chromosome elimination, (iv) formation of cytoplasmic hybrids (cybrids) to transfer cytoplasmic male sterility to a different line or species or (v) organelle transfer which is more readily achieved through fusion rather than direct uptake.

The major problem, after the fusion is induced between genetically different lines is the isolation or selective growth of the heterokaryons in the midst of parental cell population and homokaryotic fusion products. This could be achieved by precisely picking up the heterokaryons by micromanipulators or isolation through more sophisticated florescence flow cytometer or cell sorter and to grow them in nurse culture. But the selective method of culture for heterokaryons is more widely used. This is achieved by standardising culture media which is only conducive to the growth of the somatic hybrid cells but not to the parental cells lines. This type of selective method becomes possible when double-albino, auxotrophic and drug or antibiotic resistant mutants are available and wild types are formed by complementation through hybridization which are automatically selected. However, in the absence of such double mutants, half selective method is taken recourse to and in quite a number of cases, hybrid colonies are easily identified in a mixed culture. Basically for the identification of two parental cell lines in the fusion process chlorophyllose mesophyll

Contd...P/5.

protoplasts (fluorescing red under UV) are directly taken from the leaves of one parent and colourless protoplasts (florescing green under UV if treated with FDA or FITC) taken from cell suspension of other parent maintained for long time through cell passages. After the hybrid colonies are isolated they are grown separately and plantlet regeneration is induced following the basic techinques of tissue culture. When full fledged plants from the fusion products are raised and they bear flowers and fruits, various tests such as morphological, cytological and biochemical are carried out to prove the authenticity of their hybridity. Quite often fused products may not yield hybrid plants due to break-down at various stages.

Though a few years back somatic hybridization experiments were limited only to certain model plant systems predominantly from Nicotiana, Petunia, Solanum, Datura, Daucus (Schieder, 1982) etc. gradually these are being extended to other plant genera such as Lycopersicon, Oxyza etc.

- 0 -

Complex Review

THE TISSUES OF THE PLANT BODY

Dr. G.V. Gopal
Lecturer in Botany.

Meristems :-

As a general rule, one of the obvious differences between plants and animals is the pattern of growth by which they attain their adult forms. Animals grow until they reach physical maturity, and during this time growth occurs throughout the body. Plants, however, continue to grow until they die but, as soon as the embryonic stage is passed, growth is restricted to certain regions called meristems (Fig.3.1). Here the cells remain embryonic and continue to divide, whereas in the rest of the plant they reach maturity and assume a permanent form. One type of meristem is always present at the tip of every root and stem. The activity of such apical meristems is responsible for the increase in length of the plant body and, in the case of shoot meristems, for the production of lateral branches, leaves and flowers. The growth initiated by apical meristems is known as primary growth, and all tissues formed from apical meristems are called primary tissues. In some plants, notably grasses, the increase in length of the stem is also due to the division of meristematic cells located at the base of each node and leaf sheath. These meristematic regions are called intercalary meristems (Fig.3.1b) because they are inserted or intercalated between mature primary tissues both above and below them. They are really portions of the apical meristem which become separated from the main body of the meristem and are left behind as the apex grows forward. Intercalary meristems may remain active long after the cells of the internodes above them have fully matured. Growth of the cells produced by intercalary meristems is responsible for the rapid elongation of the stem which often occurs just before flowering. The tissues formed from intercalary meristems are similar to the adjacent tissues derived from the apical meristem and are therefore classified as primary tissues.

Contd...P/2.

Some plants, especially monocotyledons, complete their life cycle by primary growth. In other plants, including most dicotyledons, the stem and root increase in thickness by means of a process called secondary growth which is initiated by lateral meristems or cambia (singular, cambium). These develop within the already existing primary tissues of the root and stem, and form secondary tissues in planes parallel with the surface of these organs (Fig. 3.1a). There are two cambia that may develop in a plant showing secondary growth, the vascular cambium and the phellogen (or cork cambium). The vascular cambium is responsible for most of the increase in thickness during secondary growth, whereas the phellogen produces a protective layer of periderm (or cork). This is formed in the outer region of the expanding root or stem when the primary surface layer (epidermis) is ruptured by the increase in thickness due to the activity of the vascular cambium.

Maturation of meristematic cells :-

Since all the cells of the adult plant body result from the activity of meristems, the question arises as to how meristematic cells are converted into mature cells. The essential characteristic of a meristematic region is that it consists of actively dividing cells which have the dual property of maintaining the meristem as a distinct region and, at the same time, of adding new cells to the rest of the plant body. In every meristem there are certain cells which divide in such a way that at each division one of the daughter cells (the initial) remains in the meristem, whereas the other daughter cell (the derivative) gradually passes out of the meristem and eventually becomes one or more cells within the main body of the plant. The stages by which a newly formed derivative reaches maturity can more easily be followed by reference to apical meristems rather than to lateral meristems.

Contd...P/3.

The meristematic cells at the apices of roots and stems (but not those of lateral meristems) have the characteristic appearance shown in Fig. 3.2. Although they appear approximately square in section, their basic shape is that of a 14-faced polyhedron, each face pentagonal. This is the shape assumed when a number of similar clastic spheres are subjected to pressure equally from all directions, until there are no air spaces between them. The cells have thin walls, the cell cavity is filled with dense cytoplasm and a large nucleus (up to two-thirds the diameter of the cell), and visible vacuoles are absent. During the process of becoming transformed into a mature cell, a derivative passes through three distinct but overlapping phases. The ability to divide is not confined to the initial cells but extends also to their immediate derivatives, which usually divide several times before starting to mature. The first phase in the growth of a derivative is therefore cell division, but this soon passes into the second phase, namely cell enlargement. Because the apical meristem constantly grows forward as a result of cell division, the newly formed derivatives come to occupy the region just behind the apex. It is in this subapical region that they begin to enlarge, and they continue to do so until by the time they reach maturity they are commonly many times larger than the meristematic cells from which they were derived (Fig. 3.2).

As a cell enters the phase of enlargement, droplets of cell sap form in the cytoplasm and these, after further increase in size, fuse to form several small vacuoles. The nucleus meanwhile remains suspended in the centre of the cell by strands of cytoplasm. The vacuoles continue to enlarge by uptake of water and finally coalesce to form a single central vacuole. As a consequence, the nucleus is moved to a peripheral position in the thin layer of cytoplasm lining the cell. While the vacuoles are expanding the whole cell increases in size but, despite the increase in surface area, the cell wall does not decrease in thickness because new wall material is continually being added to the original wall. Thus cell enlargement is not merely the inflation of a cell

Contd...P/4.

due to vacuolation, but it is an active process in which the amount of plant substance is increased as a result of intense biochemical activity by the cell.

In the process of assuming their mature form, cells not only increase in size but also become structurally modified to fulfil particular physiological functions in the adult plant. This modification for specialized functions is called differentiation (Fig. 3.3) and forms the third and last phase in the maturation of a meristematic cell. Differentiation starts while cells are still enlarging but is never complete until after they have ceased to enlarge. It should be regarded as the process by which cells become different not only from their meristematic precursors but also from their immediate neighbours. The extent to which a cell becomes differentiated depends on its final function. Some cells differ only slightly from meristematic cells (e.g. packing cells) while others become markedly different (e.g. elongate conducting cells). There are many ways in which plant cells can become specialized to serve particular functions, but most of them involve modifications of the cell wall. These modifications include such features as the deposition of a thick secondary wall, and the development of various types of pits.

Types of plant tissues :-

It should now be clear that the different types of cells in the adult plant are the product of the three overlapping processes of cell division, cell enlargement and cell differentiation. The resulting mature cells are not arranged at random but are associated in various ways to form recognizable groups called tissues. There are various ways of classifying tissues according to whether they are considered from a structural or functional point of view. Perhaps the simplest classification, and the one that will be followed here, is to divide tissues into two categories, simple and complex, on the basis of whether they consist of only one or more than one type of cell.

Contd...P/5.

Simple tissues :-

There are three simple tissues, parenchyma, collenchyma, and sclerenchyma. These terms are also applied to individual cells showing the characters of these tissues. Thus a parenchyma cell is not necessarily a unit of the simple tissue parenchyma, but it may also be a component of a complex tissue.

Parenchyma

This is the simplest type of mature tissue, being less modified from meristematic cells than any other tissue. Parenchyma cells are living cells which are sufficiently unspecialized to be capable of reverting to the meristematic condition. It is often difficult to draw sharp lines between tissues because cell types sometimes merge into one another. Parenchyma, in particular, has very ill-defined limits and covers a range of cells which differ widely in structure and function. It is thus impossible to say that a parenchyma cell will inevitably have certain features apart from the very general one of being alive at functional maturity. However, 'typical' parenchyma cells (Fig.3.4) can be expected to to be more or less isodiametric in shape, with or without intercellular spaces between them, and to have thin cellulose walls; pits, if present, are always of the simple type. Parenchyma, as its Greek derivation (literally 'poured beside') indicates, forms the basic packing tissue in which more specialized tissues appear to be embedded. Besides its packing function, parenchyma may also be concerned with photosynthesis and with the storage of stareh and other substances. In relation to these two functions parenchyma cells vary considerably both in shape and living contents. Thus the parenchyma cells of the photosynthetic tissue of a leaf are rich in chloroplasts and may be considerably elongated or lobed. While those found in food-storing structures such as a bean seed (Phaseolus) or a sweet potato (Ipomoea batatas) are packed with starch grains. In addition to forming a simple tissue, parenchyma cells are a regular component of the two

Contd...P/6.

complex tissues, xylem and phloem, where they commonly serve for the storage of various substances, particularly starch.

Collenchyma :

This is a living tissue which has many of the characteristics of parenchyma, and may indeed be interpreted as a form of parenchyma structurally specialized as a supporting tissue in young organs. When collenchyma and parenchyma lie next to each other they frequently merge into one another through transitional types. The resemblance to parenchyma is also indicated by the common occurrence of chloroplasts in collenchyma, and by the ability of this tissue to resume meristematic activity. Collenchyma occurs immediately beneath or near the surface of young stems and petioles, and along the main veins of foliage leaves; it is rarely found in roots. The cells of collenchyma (Fig. 3.5) are elongated in the direction of the long axis of the organ in which they occur, and are characterized by the presence of thick, non-lignified, primary walls. The wall thickening, however, is not uniformly deposited around the inside of the cell but is thickest in the corners of the cell (angular collenchyma), although the two tangential walls may also be thickened (lamellar collenchyma). In longitudinal section therefore collenchyma shows thin and thick portions depending on the plane of the cut (Fig.3.5b).

The thick cellulose walls of collenchyma combine considerable tensile strength with plasticity. Hence collenchyma is particularly sutiable for the support of actively growing organs because its cells can extend to keep pace with the elongation of the organ and yet retain their strength.

Sclerenchyma :

Whereas collenchyma is the main supporting tissue of actively growing organs, sclerenchyma performs a similar function in mature plant parts. Sclerenchyma cells have thick secondary walls, usually lignified, and their protoplasts are dead, or at any rate inactive, at maturity.

Contd...P/7.

Sclerenchyma is a very variable tissue but two broad categories of it can be recognized, fibres and sclereids; in general fibres are very much longer than sclereids.

Fibres Fibres are long narrow cells with tapering pointed ends (Fig.3.6). They are usually massed together in long strands, their tapering ends overlapping one another and fitting tightly together. While fibres are young and actively growing, their end walls tend to slide over one another to produce the pointed state of the mature fibre. The increase in length of a fibre by the intrusion of its growing tips between the walls of neighbouring cells is called intrusive growth. After a fibre has ceased to elongate, its walls continue to thicken until at maturity the cell cavity is very much reduced, and sometimes almost obliterated. Fibres occur in most parts of the plant body and, according to their position, they may be classified into two types: xylary fibres which occur in the complex tissue xylem, and extraxylary fibres which occur in any tissue other than xylem. Xylary fibres have reduced bordered pits, the inner apertures being slit-like and those of a pit-pair often crossed with each other. Extraxylary fibres have very narrow, simple pits. Although the thick secondary walls of fibres are usually lignified, some fibres have unmodified cellulose walls (e.g. those of the flax plant (Linum usitatissimum), from which linen is made). Xylary fibres are a major component of wood, and on account of their heavily lignified walls, are responsible for the hardness and rigidity of this material. Extraxylary fibres, some lignified and others not, are common sources of rope, sacking and clothing textiles.

Sclereids :-

These are very variable in shape, ranging from approximately isodiametric to very irregular, and they may even be branched (Fig. 3.6 f-h). They have very thick, lignified walls, often showing concentric layering, which are pierced by numerous simple pits. [illegible]

[illegible] small [illegible] in the flesh [illegible] or as [illegible] in [illegible]

Frequently the pits become branched as a result of the fusion of two or more pit cavities during the increase in thickness of the secondary wall, Sclereids may occur either singly or as small clusters among other cells in, for example, the gritty specks in the flesh of a guava (Psidium guajava), or as continuous masses as in the hard shell of a coconut (Cocos nucifera).

Complex tissues :-

Although a complex tissue is composed of several different types of cells it merits being recognized as a single tissue because it has certain features peculiar to itself, occurs in definite positions in the plant body, and is associated with definite functions. There are two complex tissues, the xylem which is concerned primarily with the conduction of water and inorganic solutes, and the phloem which is concerned primarily with the conduction of food manufactured in the leaves. These two complex tissues always occur side by side, and together constitute the vascular or conducting system which extends throughout the plant.

Xylem :-

The xylem is composed of parenchyma and fibres, which have already been described, and tracheids and vessel members, which are characteristic of this tissue. These four elements occur in different proportions and combinations, and all four are not necessarily present in the xylem of any given plant.

Tracheids :-

Tracheids are more or less elongated cells, which are angular in cross-section and have oblique or tapering end walls (Fig. 3.7). Tracheids are dead at functional maturity when they consist only of lignified cell walls. All tracheids have secondary walls but these are deposited in various patterns which are related to the state of maturity of the region in which the tracheid is being formed

Contd...P/9.

(Fig. 3.8). In the tracheids formed nearest to the apical meristem the secondary walls are deposited as a series of horizontal rings (annular thickening) and in those further back as one or more spirals (helical thickening). These are succeeded by tracheids in which the secondary wall forms a net-like pattern (reticulate thickening) over the primary wall, and finally by tracheids in which the secondary wall is continuous except for elongate or circular pits (pitted walls). These different patterns of secondary wall thickening form a series in which progressively more of the primary wall is covered, but the different types often merge and not all types are necessarily represented in any given plant. All four types of thickening give support to the tracheids in which they occur, so that the central cavity or lumen is kept open despite pressure from neighbouring cells. It is interesting to note, however, that tracheids with annular and helical thickenings are formed while the surrounding tissues are still elongating, and they continue to be stretched even after they are structurally mature. The occurrence of stretching is shown by the fact that, when several helical tracheids are present end to end in a longitudinal series within the region of elongation, the spirals of the older tracheids are more drawn out than those of the younger ones. Tracheids with more extensive, and therefore more rigid, wall thickening are not readily stretched, and such tracheids differentiate in plant organs which have stopped elongating.

Water moving through tracheids passes from one cell into the next through the areas, whether extensive or restricted to pits, where the primary wall only is present. Tracheids perform the double function of a supporting and water-conducting element, and in such vascular plants as ferns and conifers, which are more primitive than angiosperms, they are the only cell type present in the xylem apart from parenchyma. During the course of evolution the tracheid diverged along two routes, one in the direction of greater mechanical efficiency which resulted in the xylary fibre

Contd...P/10.

(already described), and the other in the direction of greater water-conducting efficiency which resulted in the vessel member. Tracheids, however, were not eliminated when fibres and vessel members were evolved.

Vessel members These are cylindrical cells, non-living when mature, which are joined end to end to form multicellular water-conducting tubes, called vessels. The end walls (and sometimes also the side walls) of vessel members become perforated by one or more holes through which water can pass freely from cell to cell (Fig.3.7d-f). Hence, instead of a tier of superimposed cells, there is formed a tube rather like a drainpipe in structure, which is the vessel. Vessel members are commonly shorter and much wider than tracheids, but they develop lignified secondary walls in a similar way and show the same patterns of wall thickening (Annular, helical, etc.) as tracheids.

Vessel members begin life as separate cells with a continuous primary wall, but at a certain stage in development the middle lamella swells in the areas which are to become perforated (Fig.3.9). Subsequently a secondary wall develops, but this is not deposited on the areas (e.g. primordial pits) which persist as thin primary wall or on the areas of future perforation. As the vessel members mature, the primary walls and middle lamella in the perforation areas break down, so leaving open holes. The perforated areas (called perforation plates) may be simple, with one large perforation, or multiperforate, with more than one perforation (Fig.3.7d-f,h,i). In a mature vessel with simple perforation plates the previous position of each cross-wall is often indicated by the presence of a distinct rim of thickening around the lumen of the vessel. Vessels are not of indefinite length, although they frequently extend for several metres. Where a vessel ends, the terminal vessel member is perforated at its proximal end but not at its distal end. Therefore the passage of water from vessel to vessel takes place through the areas of primary wall (such as pits) in a manner similar to conduction in tracheids.

Contd...P/11.

In transverse section vessels can usually be recognized by their large, almost circular outlines. Whereas the widest tracheids scarcely exceed 100 um in diameter and most are much narrower, vessels are commonly 300 um across and some, especially those of climbers, reach a width of 700 um. The wide diameter of vessels, coupled with the perforations in their end walls, enables water to be conducted more efficiently in these elements than in tracheids.

PHLOEM

The characteristic components of the phloem are sieve-tube members and companion cells, but parenchyma and, in some cases, fibres also occur.

Sieve-tube members These are elongated cells joined end to end into sieve tubes (Fig.3.10) in a manner analogous to the joining of vessel members to form vessels in the xylem. Unlike vessel members, however, the cell walls of sieve-tube members are not lignified and are classed as primary walls, even though they are usually thickened. Also unlike vessel members, the cross-walls of sieve-tube members, although much modified, do not break down completely. Instead, the cross-walls become perforated by open pores through which the protoplasts of two superimposed members are continuous. These connecting strands of cytoplasm are comparable with plasmodesmata but very much wider. The perforated cross-walls are called sieve plates because in surface view they resemble sieves (Fig.3.11). It is from the presence of sieve plates that this characteristic cell type derives its name. Usually the sieve plate covers the entire cross-wall, but sometimes it is broken up into several perforated sieve areas, thus forming a compound sieve plate.

In the sieve plate each cytoplasmic strand is enclosed in a cylinder of a special kind of carbohydrate called callose. Aniline blue
The thickness of this callose cylinder obviously affects the size of the sieve pore. When the plant is dormant thick callose pads completely block the pores, but at the onset of

Contd...P/12.

the growing season much of the callose is removed and new connecting strands of cytoplasm appear. If the plant is injured callose is also deposited, often within a few seconds of the injury, thus blocking the pores and minimizing loss of food material. One consequence of this instantaneous reaction is that in most microscopic sections the pores are completely blocked. This is the result of the treatment of the tissue for examination, and does not represent the situation in an actively growing plant.

Sieve-tube members are living cells and only function as long as they are alive. A remarkable characteristic of sieve-tube members is that during development the nucleus that was originally present breaks down and completely disappears at maturity. They are the only known example of plant cells which are living but lack a nucleus. Investigation with the EM has indicated that sieve-tube members are also remarkable in possessing very few mitochondria and almost no other cytoplasmic organelles. Furthermore, the normal distinction between vacuole and cytoplasm seems to be lacking, the main volume of the cell being occupied by a fibrillar protein, called P-Protein, whose exact structure and function is currently the subject of active debate among plant physiologists. The proteinaceous material readily takes up staining reagents, and in stained longitudinal sections usually appears attached to the sieve plates as so-called slime plugs. The presence of slime plugs provides one of the most useful criteria for identifying the position of phloem in longitudinal sections (Fig.3.10a).

Companion cells Each sieve-tube member is closely associated with one or more slender parenchyma cells, called companion cells, which have dense cytoplasm and conspicuous nuclei. Companion cells arise by an unequal longitudinal division of the same mother cell as the sieve-tube member (Fig.3.12). After being cut off from the sieve-tube member the companion cell does not necessarily remain as a single cell but may divide horizontally into a vertical file of

Contd...P/13.

(conducting water) and the phloem (conducting food). Becau of the presence of fibres in them, the vascular tissues, particularly the xylem, are also concerned with support. Th ground system includes the tissues that can be regarded as forming the ground substance in which the vascular tissues are embedded. The main ground tissues are the three simple tissues, parenchyma, collenchyma and selerenchyma.

Within the plant body the three tissue systems are distributed in characteristic patterns depending on the orga in which they occur, on the taxonomic group to which the plant belongs, or on both (Fig.3.13). Since the dermal syste is always superficial, the principal differences in pattern depend on the relative distribution of the vascular and ground systems. In a dicotyledonous root, for example, the vascular tissue usually forms a central rod embedded in the ground tissue (cortex). In a dicotyledonous stem, however, the vascular tissue typically forms a hollow, perforated cylinder with some ground tissue (pith) enclosed within the vascular cylinder and some located between the vascular cylinder and the dermal tissue. The latter region of ground tissue is called cortex because, like the ground tissue in the root, it occurs outside the vascular tissue. This method of interpreting the anatomical pattern of an organ is not only descriptively useful, but also reflects the basic unity underlying the organization of the plant as a whole.

Whatever the arrangement of tissues in a mature organ may be, the pattern is established immediately behind the apical meristem from which it is derived. In this subapical region, where the cells are still actively dividing. It is thus possible to recognize three types of meristematic tissue. The protoderm, ground meristem and procambitum. These are sometimes called the three primary meristems because they are the precursors of the dermal,ground and vascular tissue systems respectively. Although leaves originate on the flanks of the shoot apex, these three types of meristematic tissue are also present in a developing leaf.

Contd...P/15

Table 8.1 Characteristic features, funtions and distribution of plant tissues.*

Tissue	Living or dead	Wall material	Cell shape	Main functions	Distribution
Parenchyma	Living	Cellulose, pectins and hemicelluloses	Usually isodiametric sometimes elongated	Packing tissue support in herbaceous plants. Metabolically active. Intercellular air spaces allow gaseous exchange. Food storage. Transport of materials through cells or cell walls.	Cortex Pith, Meduallary rays and packing tissue in xylem and phloem
Modified Parenchyma					
a) epidermis	Living	Cellulose, pectins and hemicelluloses and covering of cutin	Elongated and flattened	Protection from desiccation and infection. I lairs and glands may have additional functions.	Single layer of cells covering entire primary plant body.
b) mesophyll	Living	Cellulose, pectins and hemicelluloses	Isodiametric, irregular or column-shaped depending on location	Photosynthesis (contains chloroplasts). Storage of starch.	Between the upper and lower epidermis of leaves
c) endodermis	Living	Cellulose, pectins and hemicelluloses, and deposits of suberin	As epidermis	Selective barrier to movement of water and mineral salts (between cortex and xylem) in roots, Starch sheath with possible role in geotropic response in stems.	Around vascular tissue (innermost layer of cortex)
d) pericycle	Living	Cellulose, pectins and hemicelluloses	As parenchyma	In roots it retains meristematic activity producing lateral roots and contributing to secondary growth if this occurs	In roots between central vascular tissue and endodermis

Contd...P/16.

Collenchyma	[illegible]	[illegible] hemicelluloses	Elongated and polygonal with tapering ends	Support (a mechanical function)	Outer regions of cortex e.g. angles of stems, midrib of leaves
Sclerenchyma a) Fibres	Dead	Mainly lignin, Cellulose, pectins and hemicelluloses also present.	Elongated and polygonal with tapering interlocking ends	Support (purely mechanical)	Outer regions of cortex, pericycle of stems, xylem and phloem.
b) sclereids	Dead	As fibres	Roughly isodiametric though variations occur	Support or mechanical protection	Cortex, pith, phloem, shells and stones of fruits, seed coats.
Xylem	Mixture of living and dead cells. Xylem also contains fibres and parenchyma which are as previously described.				
tracheids and vessels	Dead	Mainly lignin, cellulose, pectins and hemicelluloses also present.	Elongated and tubular	Translocation of water and mineral salts. Support	Vascular system
Phloem	Mixture of living and dead cells. Phloem also contains fibres and sclereids which are as previously described.				
a) sieve tubes	Living	Cellulose, pectins and hemicelluloses	Elongated and tubular	Translocation of organic solutes (food)	Vascular system
b) Companion cells	Living	Cellulose, pectins and hemicelluloses	Elongated and narrow	Work in association with sieve tubes	Vascular system

* Tissues associated with secondary growth, such as wood and cork, are described in chapter 21.

PLANT - TISSUES
PRACTICAL

Dr. G.U. Gopal
Lecturer in Botany
RCE, Bhubaneswar

APPENDIX - I

The following brief notes on certain phases of microtechnique are given here to facilitate the use of this book by the teacher and student. For full information on the various procedures used in preparing tissue for microscopic study, reference should be made to the publications of Chamberlain (1932), Rawlins (1933), Johansen (1940), and Sass (1940), [illegible] General References.

FREE-HAND SECTIONS

In many of the exercises in this book, directions are given for the study of sections cut by hand from living stems, leaves or other plant structures. To prepare such material requires only simple technique and in addition provides a realistic picture of cells and tissues that should precede the examination of microtomed and permanently stained preparations. In the laboratory the student can acquire the necessary skill with a sectioning-razor to enable him to explore the structure of such tissues as the epidermis, parenchyma, collenchyma, and the phloem and xylem of vascular bundles. Best results are secured by enclosing small portions of the material between the split halves of pieces of elderberry pith or of carrot root. Often it is necessary to make a groove in the inner surface of one of the pith halves or strips of carrot to accommodate such bulky objects as stems, petioles, etc. In sectioning, hold the object enclosed between the pieces of pith at as constant an angle as possible and transfer each section to water; the slices of pith can be removed by the use of a brush. Sections cut by hand should be carefully mounted on a clean slide either in distilled water or in the various reagents designated and the cover-glass lowered gently into place. For more resistant cells, such as sclereids or fibers and for the critical study of the sieve-plates in phloem elements, the use of the carbon dioxide freezing microtome is highly desirable. With the aid of this

Contd...P/2.

instrument a large number of thin sections may be prepared by the instructor in advance of class use (for a description of the technique of sectioning with the freezing microtome, cf. Sass, 1940, pp. 97-98). The student must learn to check free-hand preparations at frequent intervals so that the sections are not allowed to dry out. Cells immersed in fluid are not only easier to study from an optical point of view, but they also retain a more or less normal structure over a relatively long period of observation. Sections of hairy objects, such as many leaves or stems, are often difficult to mount in water without the formation of numerous air-bubbles. This difficulty may be removed by mounting each section in a weak solution of alcohol. This acts as a killing reagent for the protoplasm, but it does make possible the accurate study of the shape, arrangement, and character of the walls of cells.

PREPARED SLIDES

The use of permanent slides is essential in the study of many of the topics outlined in this book. This is particularly true for the work to be done under Exercises III, X, XII, XIII, XIV, and XV. Suitable preparations as a basis for class study are obtainable from commercial supply houses or may be prepared for the student directly. With reference to the latter possibility, detailed suggestions for the collection, fixation, sectioning, and staining of tissues and organs are presented systematically in the recent manuals on microtechnique by Johansen (1940) and Sass (1940).

MACERATED TISSUE

One of the most important skills that the student must develop in laboratory practice is the ability to visualize cells as three-dimensional bodies. This is often extremely difficult on the basis of the examination of sections that tend to create a two-dimensional concept. Furthermore, many definitive features of cells, particularly the structure and arrangement of pits and the varied secondary wall thickenings in tracheary

Contd...P/3.

elements the character of perferations in vessel elements and the form of ramified selereids, can best be studied in isolated cells. For these reasons, a study of macerated tissue is recommended for many topics in this book and is especially desirable in connection with Exercises II, VIII, IX, and X. The maceration of plant tissue is most effectively accomplished by the use of certain reagents that dissolve the intercellular substance and thus cause the separation of a piece of tissue into its component cells. Jeffrey's method is usually satisfactory, especially for hard lignified structures. Small pieces of the material, no thicker than a match, are placed in a glass vial containing a mixture of equal parts of 10% chromic acid and 10% nitric acid. The vial is then corked and placed in an electric oven at a temperature of 30°-40° C. until the material becomes soft or "mushy" in texture. Hard material, such as wood and the shells of nuts, may require several days in the oven, during which time it is advisable to change the macerating fluid once or twice. Boiling small slivers of wood before placing them in the acids drives out the air and accelerates the maceration process. It is advisable to stop the action of the macerating fluid before a complete separation of all cells has occurred. Small pieces of "mushy" tissue, placed in water on a slide, can be teased apart with needles; such a procedure yields very instructive preparations of connected cell groups as well as isolated elements. The macerated tissue is carefully washed in distilled water to remove as much of the acid as possible and can them be transferred to 50% alcohol for future study. Often effective results may be secured by staining the isolated cells in safranin. Permanent preparations of macerated tissue are easily made by placing small quantities of cells in water on a slide, evaporating the excess water on an electric hot-plate and mounting in glycerine jelly. Circular cover-glasses should be used, the edges of which can be sealed with some type of cement which prevents drying out and the entrance of air.

For some tissues, for example, the cortical and pith parenchyma of herbaceous stems and the mesophyll of leaves, the following more gentle maceration technique should be employed. Place small portions of the tissues in acid-alcohol (3 parts of 70% ethyl alcohol; 1 part concentrated HCI) and thoroughly

Contd...P/4.

remove the air by means of an aspirator. Add fresh acid-alcoh and allow this to act on the tissue for 24 hours. After thoro washing in water, transfer the tissue to a 0.5% aqueous soluti of ammonium oxalate. Within a few days (or sooner depending u the material) the parenchyma or collenchyma tissues can readil be dissociated, by gentle teasing with needles, into their component cells. This method is highly recommended for isolati the idioblastic ramified sclereids of such plants as Camellia, Osmanthus, etc., from neighbouring tissue elements.

Stain Preparation

1.	Safranin	: Dissolve 1 gm of dye in 50 per cent alcohol.
2.	Fast green	: Dissolve 1 gm of dye in 90 per cent alcohol.

Mounting

All the stained thin sections are transferred to drop of Canada Balsam (mounting media) taken on the slide. Covered with cover slip a permanent stained slide lasts longer.

IX Maceration

Sections of the plant parts do not depict the actual nature of the constituent cells. The cells can be studied by dissociation method called maceration. The materials are treated with chemicals which dissolve the middle lamella of the cell wall. This also dissociates the cells from one another. The tissues like xylem and phloem depict the actual nature of the constituent cells.

Method

Cut the material into small pieces and treat it for 24 hours with acid alcohol solution (HCI, alcohol). Wash the materials and transfer to 0.5% ammonium oxalate solution and boil. Mount the tissue on the slide in drop of above solution and study.

Macerating Fluid

1.	Ammonium Oxalate Fluid	
	Hydrochloric acid	30 cc
	Alcohol 95 per cent	100 cc
	Ammonium oxalate	0.5 per cent

Contd...P/

2. Schule's Fluid

Potassium chlorate	1 gm
Nitric acid	50 cc

The materials are heated in the mixture for sometime, washed and mounted in glycerine.

CLEARING TECHNIQUE

It is extremely laborious and in many cases unnecessary to attempt to reconstruct the gross anatomy of the vascular system of a flower or the venation of a leaf by means of serial sections. The following procedure yields very instructive preparations and is applicable to herbarium specimens as well as to fresh material.

1. Leaves. Small leaves can be cleared in toto. Large laminae, however, should be subdivided into strips (about ¾ inch in width) extending from the mid-rib to the margin. The leaf material of herbarium specimens should first be soaked in hot water until it sinks; with fresh leaves, the chlorophyll should be extracted by means of hot alcohol. Following these preliminary treatments, the material is then transferred to vials containing a 5-10% aqueous solution of NaOH. With delicate objects, the removal of the cell contents may be achieved without heat. But for thick, coriaceous leaves, the vials should be placed in an electric oven. Frequent changes of the reagent are desirable until the leaf material appears devoid of discoloration. After washing thoroughly in distilled water, the material should be examined (without a cover-glass) under low magnification; in many cases the stomata, veinlets, and other histological features (e.g., idioblastic sclereids) can now readily be studied. Cleared material at this stage can be stored for future examination in 50% alcohol. Very often, however, even after several days treatment with NaOH, the material may still be opaque. In such instances, dehydration in successive grades of alcohol followed by clearing with xylene or toluene are necessary. Syracuse watchglasses are convenient receptacles and the following series is recommended: 50% alcohol, 95% alcohol, 100% alcohol (2 changes), 100% alcohol-xylene, pure xylene. Use a camel-hair's brush or

Contd...P/6

forceps in transferring the material through this series, draining off the excess fluid on filter paper at each change. Because of the large volume of tissue represented, dehydratio must be very thorough or a milky turbidity will form when the final transfer to xylene occurs. To avoid this, allow the material to remain in each of the grades of alcohol for at le 10-15 minutes. After several pieces of leaves have passed through the entireseries, the reagents should be discarded an fresh alcohol and xylene added to the watch-glasses. To make permanent mounts, transfer a piece of material to a clean sli flooded with xylene, add a generous amount of balsam and carefully lower a cover-slip into place. The slides should then be placed on an electric-slide warmer for a week or longer until thoroughly dry. Various types of stains (e.g. safranin, Delafield's hematoxylin) often increase the usefulness of cleared leaf-material. These stains may be introduced at various points in the alcohol-xylene series.

2. Flowers. The procedure outlined above is also applicable to the study of the vasculation of the floral axis and its appendages. In making permanent mounts of cleared flowers it is often desirable to use "depression slides"; these permit the inclusion under the cover-slip of relatively thick objects.

SPECIAL REAGENTS

1. Phloroglucinol and hydrochloric acid. The addition of these reagents produces a red color in the walls of sclereids lignified fibres, and trachеary elements (of Exercises VIII, IX and X). The stain it not permanent but nevertheless is extremely useful in demarcating the thick walls of certain types of cells. A saturated solution of phloroglucinol should be prepared in 18% HCL. Mount the section or tissue fragment directly in a drop of this reagent on the slide and add a cover-slip. Great care should be taken to carry out this procedure some distance away from the microscope.

2. Potassium Iodide (IKL) and sulphuric acid. This is a specific test for cellulose. Mount the sections in the potassium i-dide (1g. iodine and 3g potassium iodide in 300cc.

Contd...P/7

of distilled water, according to Rawlins, 1933) and add a cover-slip. The introduction of a drop of 65% sulphuric acid will cause cellulose walls to turn blue in color.

3. Anilin blue. This is a specific stain for callus depositions on sieve plates and is essential for the procedure outlined in Exercise XI. Sections should be immersed for a short time in a 1% aqueous solution, and then transferred, after gentle washing, to a drop of water. The callus on the sieve plates is stained blue. Dr. A.S. Crafts has suggested to the writer the following improvement: Place the sections in IKI, wash in water, stain in anilin blue for about five minutes, then wash briefly again with IKI and mount for study in tap water or glycerine.

4. Neutral red. This vital stain is very useful as a general stain for the primary walls of living cells, and is recommended for use in Exercises V, VI, and VII. Mount the sections directly in a 1% aqueous solution.

5. Sudan IV. This reagent is specific for the cuticle and for cutinized and suberized cell walls. Place the sections in a drop of alcoholic solution of Sudan IV (5g. in 100cc. of 80% alcohol), add a cover-glass, and examine under the microscope. The cuticle, as well as waxy materials present in walls, are stained red. This reagent is very desirable for use with Exercise V.

%%%%%%%%%%%%%%%%

The Tissue System

Dr.U.K.Nanda
Reader in Zoology

Introduction

The cells in the body of the multicellular animals vary in structure and function. It is seen that one variety of cell performs one kind of work and constitutes one type of tissue.

Definition

So a tissue may be defined as an aggregate of same types of cells i.e. similar in morphology, physiology and embryonic origin, combined by subserving the same general function independently and united by varying amounts of intercellular substance (e.g., blood, bones, cartilage, muscle, nervous tissues etc.).

Origin

The first few cells undergo repeated miototic division. The first evidence of differentiation is discernible in this cellular mass, where distinct layers, known as the ectoderm, the mesoderm and the endoderm. Cells of each of these layers reorganise themselves to form tissue system.

There are four primary types of tissues in the body:

i) Epithelial - which covers surfaces, lines cavities and forms tubes.

ii) Connective - which forms supporting and binding structures.

iii) Muscular - which comprises the contractile elements.

iv) Nervous - which makes up the regulating and conducting structures.

The Principal differences among the various types of tissues arise from the types of cells of which they are composed, the nature and amount of intercellular material present, and the functions they perform.

1) Epithelial Tissue

Forms the covering of the outer surfaces of the body and of most internal organs, lines the digestive and respiratory tracts, the serous cavities, blood vessels, excretory ducts and reproductive ducts and organs. It comprises the secreting portions and ducts of glands and the sensory portions of sense organs.

Structural characteristics of epithelial tissue

1) Epithelial cells are arranged in a continuous sheet, usually of one layer.

2) They are packed together closely with little intercellular substance.

3) Blood vessels are absent, but nerve endings are usually abundant.

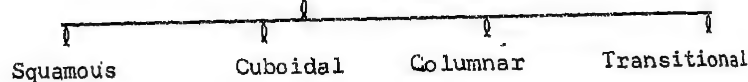
4) Epithelial cells lie on a basement membrane, a layer of intercellular substance, permeated with reticular fibers.

5) The cementing substance, here, is a mico-protein containing hyaluronic acid and calcium. salts.

Types of Epithelium

Differentiated on the basis of

(a) Shape of the cells



and (b) Their arrangement in the epithelial sheet

Simple — Stratl

According to the origin and association with other tissue it may be classified as:

Epithelium	Mesothelium	Endotheli
Form membranes associated with a thin connective tissue layer	Epithelium when derived from mesoderm e.g. peritoneum	Lines the surface of blood vess and heart

a) Based on the shape

Squamous	Cuboidal	Columnar	Transitio
Consists of cells which are thin and flat, with regular or irregular outlines	Consists of cube-shaped cells or cells in the form of truncated pyramids	Cells are long, cylindrical sometimes forming tall, irregular prisms.	Resembles s tified epit in that it sists of se layers of c but its sup ficial surf cells are l and rounded stread of b squamous in The no. of varies with condition o organ, as urinary bl

Function

1) Protection
2) Absorption
3) Secretion
4) Excretion

a) Squamous:

i) Simple (cells in a single layer) lining the body cavities and in Bowman's capsule of the kidney.

Mesothelium: that lining the serous cavities (pleural, peritoneal, pericardial)

Endothelium: that lining the blood and lymph vessels.

ii) Stratified: Squamous epithelium (cells in several layers) is found in epidermis ,cornea,oesophagus and vaigina.

b) Cuboidal:

i) Simple cuboidal: Certain glands and parts of the kidney tubules(thyroid), the ducts of glands, and the smaller bronchi.

ii) Stratified Cuboidal: Epidermisof certain tailed amphibians.

c) Columnar Epithelium: Consists of cells which are long and cylindrical, sometimes forming tall, irregular prisms.

Six Subtypes:

i) Simple : in the lining of the intestine.

ii) Simple ciliated: in the oviducts, uterus and nasal sinuses.

iii) Stratified: in the pharynx, on the epiglottis

iv) Stratified ciliated: in the larynx on the soft palate.

v) Pseudostratified: varying in height with nuclei at different levels, all cells touching basement membrane, in the parotid gland and male urethra

vi) Pseudostratified ciliated: in the trachea and Eustachian tube.

d) Transitional Epithelium

Consists of several layers of cells (like stratified epithelium) but its superficial surface cells are larger and rounded intead of being squamous in shape.

The number of layers varies with the condition of the organ as in urinary bladder.

Free surface of Epithelial cells

The free surface of epithelial cells may be modified in various ways. In some cases, the superficial protoplasm is involved; in others, a membrane-like secretion, or cuticle is formed.

Modification of superficial protoplasm

The borders of the epithelial structures may be either striated, ciliated or of the brush type.

The striated border is found in cells of columnar intestinal epithelium, usually in area where absorptive processes take place. It consists of fine striations lying perpendicular to the surface of the cells. The ciliated border is representative of respiratory epithelium cilia ar thin, very numerous, hair-like projections of varied length At the proximal end of each cilium is a small basal corpusc Ciliary movement results from the quick bending of the cili in one direction and a subsequent straightening or recovery stroke. The action of each cilium follows that of the ad-joining one in a wave-like action which is repeated. In th manner mucus or particles in contact with the cilia are mov slowly along the ciliated surface. The brush border is foun in cells comprising of certain protions of kidney tubules. consists of fine, hair-like processes which are nonmotile. gives the appearance of a dense brush.

Other specialised borders include: Stereocilia, nonmotile projections in cells of the epididymis which are thought to aid in eliminating the secretion of the cells, nonmotile hairs in hair cells of the utricle, saccule, cochlea of the ear, serving as receptors for vibratory sti Mature spermatozoa possess a flagellum, a single hair-like proceses which serves for locomotion

Structure of Cuticles

A cuticle is a layer of more or less solid substance which is secreted by and covers the free surface of an epi-thelial sheet. It is usually sharply delimited from the ce surface and capable of being separated from it.

Eg. Enamel of teeth
Capsule of the lens of the eye

Binding together of Epithelial cells

The cells of an epithelial sheet are so closely boun to one another that it usually requires considerable mecha force to separate them. The factors operating to bind them together are:

1. Interstitial substance acting as an intercellular cement.
2. Desmosomes: Condensations of cytoplasm at points contact between epithelial cells.
3. Terminal bars, rod-like thickenings of the plasma membranes which cement together adjacent cells.

Glands

Because they arise from an epithelial surface, glands are included in this discussion of the structure of epithelial tissues - sweat glads, sebaceous glands, ceruminous glands, mammary glands.

Functionally a gland is a cell or group of cells that elaborates or manufactures a specific substance which is extruded from the cell into a surface or into the blood or lymph. The product thus formed does not become a part of the body tissues but is either absorbed and used by them or discharged from the body.

Structurally - a gland is a cell or an aggregation of secreting cells. Multicellular glands arise and develop by a process of invagination, the epithelium grows into the adjacent connective tissue and a simple tube-like or sac-like structure is formed. The cells in the closed portion of the sac assume a secretory function while those near the surface narrow to form an excretory duct. The more complex multicellular glands arise by repeated invaginations, the result is a complicated, branched organ.

Secretion

Secretion is the process whereby a cell obtains materials from the blood and lymph and transforms them into products which are passed from the cell.

Polarization:

Gland cells (and, for that matter most epithelial cells) are said to be polarized; that is to say their proximal or basal end (that nearest to the basement membrane) is different in structure from the distal or free end. The nucleus lies at the proximal end, whereas, in the active phase of secretion, the secretory products(granules, mucigen or other substances) fill the distal end, from which they are discharged.

Classification of Glands

Glands may be classified on the basis of

i) the presence or absence of excretory ducts.
ii) the nature of secretion.
iii) whether the secretion is a product of the cell or a part of the cell itself.
iv) their structure.

1) Presence of excretory ducts: Based on the presence or absence of ducts the glands can be

i) Exocrine or external secreting glands: With ducts and empty their products onto a free surface(eg. salivary gl

ii) Endocrine or internal secreting glands are ductless; th secretions are absorbed into blood or lymph(e.g. thyroid gland). Some glands, for example the pancreas, ovary or tes are double-functioning, serving as combined exocrine and en crine glands.

2) Nature of secretion: Depending upon the nature of secret glands can be

Mucous glands: Secrete mucous, a viscid substance which principally contains mucin(e.g. globlet c of intestine, tracheal glands).

Serous glands: secrete a clear, watery abuminous fl (parotid gland). Salivary gl

Mixed glands : Contain both mucous and serous secretory (submaxillary gland) Salivary gl

3) Secretion of a product or a part of the cell :

Merocrine gland : The secretory product is the only par extruded (salivary gland)

Apocrine : the apical end of the cell containing accumulated products is broken off an extruded; the remaining protion of th cell is left intact, the cell reforms and the process is repeated(mammary g

Eccrine : Similar to apocrine by cytoplam is broken down before discharge.

Holocrine : The entire cell, along with its contain secretory product, is extruded, the ce is replaced by a new cell (sebaceous oil gland)

4. Classification of glands by structure:

i) Unicellular: Found on free surfaces, they secrete mucou e.g. goblet cells of intestine.

ii) Multicellular

Tubular — Albelar (acinous) — Tubuloalveolar

Tubular glands: have their secreting portion in the form of blind, narrow tube.

Simple

a) Simple straight (e.g. in large intestine)
b) Simple coiled(Sweat glands)
c) Simple branched(gastric glands)

Compound

With a large number tubes branching rep (in kidney, testis mammary glands of laying in mammal).

Alveolar glands

Have a flask-shaped secreting portion called an alveolus or acinus. They are

i) Simple (mucous and poison glands of frogs and toads)
ii) Simple branched (sebaceous glands)
iii) Compound (mammary glands)

The compound tubuloalveolar gland has a secretory portion that consists of irregularly branched tubules and saccular outgrowths (e.g. majority of endocrine glands (~~salivary~~, pancreas).

CONNECTIVE TISSUE

Dr.U.K.Nanda
Reader in Zoology

1. Definition:

Most widely distributed occurs almost in every part of the body, constitute some part of almost every organ.

2. Function

Forms the supporting and connecting structures of the body.

3. Origin

With one or two exceptions, connective tissues arise from the mesoderm, more specifically they arise from the mesenchyme of this layer.

4. Difference with epithelial tissue

i) The cells are not aggregated.

ii) A considerable amount of intercellular substance present. which is always product of the cells of the tissue.

iii) This substance is called ground substance or matrix.

iv) As the development of embryo proceeds, the nature of intercellular substance changes.

v) Fibers begin to appear, first the collagenous, then the elastic.

General characteristics of connective Tissue -

I) The cells of connective tissue are relatively few in number, the bulk of the tissue consisting of intercellular material or matrix.

ii) They are highly vascular.

iii) Rarely do they occur on free surfaces.

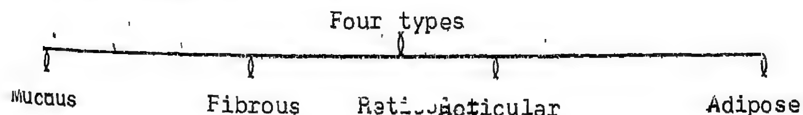
Classification of connective tissue

1) Connective tissues are classified on the basis of their intercellular substance.

i) Connective tissue proper: inter-cellular substance is of a fibrous nature.

ii) Dense connective tissue, intercellular substance i.e. rigid or semirigid (cartilage, bone)

Connective tissue proper:

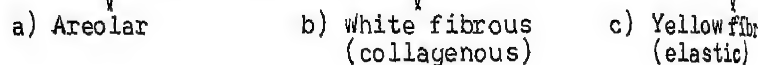


Mucous connective Tissue

1. Also known as Wharton's jelly,
2. It consists of branching cells, irregularly arranged which sometimes anastomose.
3. Jelly-like matrix present and contain mucin.
4. A few bundle of collagenous fiber may be present.
5. No elastic fibers.
6. Wharton's jelly is found only in the umbelical cord of the embryo.

Fibrous connective tissue

1. Composed of cells embedded in a matrix that is made up fibers and a semifluid substance.
2. Fibrous connective tissue is further subdivided into



a) Areolar — b) White fibrous (collagenous) — c) Yellow fibr (elastic)

a) Areolar or loose connective tissue

1) It consists of an interlacing net work of fibers in a semifluid matrix throughout which cells are scattered. forms the interstitial tissues of most
2) This kind of tissue/organs, surrounds blood vessel and nerves, and constitute most of the subcutaneous tissue and the deep fascia

b) The white fibers

1) Occur in bundles and if not stretched, are usually wavy.
2) The fibers themselves do not branch, but a bundle of them may do so.
3) They are nonelastic yet flexible.
4) They swell in weak acids or alkalies and are digested by acid pepsin but resist alkaline trypsin
5) The collagen they contain yields gelatin upon boil

c) The yellow fibers

1) Are single fibers, usually straight but containing elastin, are highly elastic.
2) They may branch and anastomose
3) Resist boiling water, acids and alkalies and are digested slowly by both pepsin and trypsin.

Histology of Aerolar tissue

The cells of areolar tissue are :

1. Fibroblasts: The cells of areolar tissue are numerous, irregularly shaped phagocytic, thought to give rise to fibers.

2. Histiocytes: (fixed macrophages) irregular in shape with branching processes and small nuclei, stain deeply, normally quiescent but actively amoeboid and phagocytic during inflamatory process.

3. Plasma cells: Smaller than histiocytes, fewer in number, function unknown.

4. Mast cells: Occur in varying numbers, found especially along blood vessles. Origin and function are not definitely known.

   Produce heparin, an anticoagulant.

5. Miscellaneous cells: eosinophils, pigment cells, undifferentiated mesenchymal cells and lymphoid "Wandering" cells.

White fibrous connective tissue

1. Consists mainly of white fibres with few elastic fibres present.

Occurrence: It is found in the fibrous capsules of organs, tendons and aponeuroses.

Tendon: A tendon is a flat or cord-like band that serves to attach muscle to bone. Its collagenous fibres are arranged in parallel bundles.

Fibroblasts; the only cells present, are arranged in parallel rows between the bundles.

An aponeurosis is a flat sheet of connective tissue that attaches muscles to bones or other tissue; it is similar in structure to a tendon.

A ligament also is similar to a tendon in structure except that its fibres are less regularly arranged and some elastic fibres may be present.

Yellow fibrous or elastic connective tissue

It is made up of principally yellow elastic fibers with a few collagenous fibers. It is found in some ligaments and in the walls of large blood vessles, especially the aorta and the larger arteries.

- It is composed of scleroprotein called elastin, seen in areolar tissue, in ligaments between joints and vertebrae.

Reticular connective tissue

Reticular connective tissue consist of a syncytial network of cells with many argyrophilic intercellular fibers running in all directions and forming a reticulum. These fibers are so named because of their property of staining intensely with

certain silver methods. This types of tissue forms the framework of lymphoid organs such as the spleen, lymph nodes and bone marrow. It is also found in certain endocrine glands in the walls of blood vesseles, and in digestive and respiratory passages underlying mucous membranes.

Adipose tissue

1) Adipose tissue is made up principally of cells that have the capacity for taking in fat and storing it

2) Mature fat cells contain a large droplet of neutral fat occupying the major portion of the cell.

3) The cytoplasm is reduced to a thin layer surrounding the droplet.

4) The nucleus is flattened and pushed to one side, giving the fat cell a "signet ring" appearance.

5) Groups of fat cells are separated by areolar tissue

6) Adipose tissue is found in the superficial fascia under the skin, around organs such as kidney, bladder, and heart, in mesenteries and the greater omentum, and as individual cells or in small groups in any loose connective tissue especially that along the blood vessels and nerves.

Functions

1) Serves as a reservoir of reserve food.
2) Protects the organs it surrounds against cold (or heat loss).
3) Protects against mechanical injury
4) Helps to support and hold organs in place.
5) Fills in the angular areas of the body.

Dense Connective Tissue:

1. This tissue includes a) Cartilage and b) bone osseous tissue

a) Cartilage: Forms foundation of endoskeleton in all vertebrates and persists in this condition throughout the life and in others partially at least in some vertebrates or wholly replaced by bone

There are three types of cartilage

i) Hyaline
ii) Fibrous
iii) Elastic

Hyaline Cartilage:

1) Consists of cells lying in cavities(lacunae) surrounded by a homogenous matrix or dense, semirigid intercellular substance.

2) The matrix contains collagenous fibers which, however, cannot be seen in ordinary preparations.

3) The cells called condrocytes, are usually single although they may occur in pairs or in groups of three or four.

4) Hyaline cartilage is flexible and slightly elastic.

5) It is covered by a membrane, the perichondrium, except over articular surfaces of the bone.

6. Hyaline cartilage is found in the articular cartilages isxfoundxinxthexarticular covering the ends of bones at joints, in costal cartilages betweenxat between the ribs and sternum, and in the septum of the nose and cartilages of the larynx and trachea.

Fibrous Cartilage:

1) Consists of a matrix not quite so dense as that of hyaline cartilage and containing many collagenous fibers that are arranged more or less in rows.

2) It possesses great strength and st flexibility.

3) The cells, avoid and enclosed in capsules, are relatively few in number and usually are widely separated.

4) This type of cartilage is found in intervertebral disks between the bodies of vertebrate and in the pubic symphysis.

Elastic Cartilage

1) It consists of a matrix having many elastic fibers f forming an interlacing net work.

2) Collagenous fibers are present but not readily seen.

3) The cells, usually single but sometimes in pairs, lie in cavities called lacunae.

4) This type of cartilage is flexible and elastic.

5) It is found in the cartilage of the external ear, the wall of the Eustachian tube, the epiglattis and certain layngeal cartilages.

Bone (Osseus tissue)

1) Bone is a rigid tissue consisting of bone cells embeded in matrix that is impregnated with calcium and phosphorous salts.

2) The structural unit is the Haversian system, which consists of cylinders of matrix and bone cells surrounding a cavity, the Haversian canal.

3) This canal contains blood vessels, lymphatics and nerves.

4) The matrix is arranged about each Haversian canal in concentric layers called lamellae.

5) Between the lamellae are lacunae, within which lie the bone cells (between osteocytes)

6) Many minute canals called canaliculi extend from each lacuna and penetrate the matrix in all directions, connecting the various lacunae.

PUMPING ACTION OF HEART

by Dr. J. K. Panda

The primary function of the cardio-vascular system is to provide an adequate supply to all cells of the body, of materials needed for their proper function and that carries away the waste products of their metabolism. It is a well-organised transport system of the body by which the blood being circulated within a closed system under different pressure gradients, created by the pumping mechanism where heart acts as the central pump. The volume of blood in our bodv is limited, but it has to perform unlimited amount of work continuously. This naturally leads to the conclusion that the same quantity of blood must be used over and over again. In other words, blood must circulate.

Blood gets reduced in the tissues and oxygenated in the lungs. Consequently, it has to pass alternately through lungs and tissues, doing opposite functions at these two places. Hence, circulatory system has been divided into two functionally opposite parts: (1) The systemic circulation (Greater circulation with high resistance circuit) - passing through the tissues. (2) The pulmonary circulation (Lesser circulation with low resistance circuit) - passing through the lungs. The two systems again meet in the heart.

ANATOMICAL CONSIDERATIONS OF HEART. The heart is roughly heart-shaped structure and rests obliquely in the thoracic cavity. The anterior surface of the heart faces the sternum, the posterior surface - the base of the cone faces the vertebral column and the inferior or diaphragmatic surface rests on the diaphragm. The heart has got four chambers two ventricles and two atria - both right and left. The two left chambers are separated from the two right ones, by a continuous partition, the atrial portion of which is called the interatrial septum (fibrous) while the ventricular part is known as the interventricular septum (upper one-fourth fibrous, lower three-fourths muscular). From the left ventricle arises the aorta, carrying

Contd...P/2

oxygenated blood to the tissues. From the right ventricle, which is less muscular than the left, arises the pulmonary trunk, carrying reduced blood to the lungs. The right atrium receives all the venous blood from the body through three veins; the inferior and the superior venae cavae, and the coronary sinus. The left atrium receives all the oxygenated blood from the lungs through pulmonary veins.

Thus the four chambers of heart perform four different functions. The course of circulation is as follows. The left ventricle propels oxygenated blood to the tissues. Here, it gives up oxygen and becomes reduced. The reduced blood comes back to the heart through the veins and is received by the right atrium. From the right atrium it passes into the right ventricle, which then propels it into the lungs. Here, it becomes re-oxygenated, and is returned to the left atrium through the pulmonary veins. From here it enters the left ventricle and is pumped out into the greater circulation again. In this way circulation goes on. Two technical terms are used in connection with heart, e.g., systole and diastole. The term systole means contraction and diastole means relaxation.

VALVES OF THE HEART

There should not be any admixture between arterial and venous blood. In other words, circulation must be strictly must be strictly one way. This is done by the action of valves. There are four sets of valves in the heart. The right atrioventricular opening is guarded by tricuspid valve the left opening by the mitral or bicuspid valve. The openings of the aorta and pulmonary artery are guarded by semilunar valves (three cusps).

The cusps of tricuspid valve and mitral valve are triangular in shape and are attached at their bases to the margins of fibrous connective tissue encircling the atrio-ventricular orifices. These valves open when the blood passes from the atria to the ventricles. The apices of the valves are projected within the ventricles during flowing of blood into ventricles. But the apices are restricted to bulge within the atrium during ventricular contraction by the presence of chordae

Contd...P/3

tendineae which attach the apical end of the valve and the papillary muscle in the ventricular wall at the other. The larger and stronger aortic semilunar valve, guards the orifice between the left ventricle and the systemic aorta, whereas the pulmonary (pulmonic) semilunar valve guards the opening between the right ventricle and the pulmonary trunk.

ACTION OF THE VALVES.

The atrioventricular (A.V.) valves open towards the ventricles and close towards the ventricles and close towards the atria. The semilunar (S.L.) valves open away from the ventricles and close towards the ventricles. So that when atria contract, atrioventricular valves open and blood passes into the ventricles. When ventricles contract, atrioventricular valves close, but semilunar valves open. This prevents regurgitation of blood into the atria but allows it to flow out of the ventricles. In this way circulation becomes one way.

The two ventricles contract simultaneously, as also the two atria. The same amount of blood passes out of the ventricles at the same time during systole. The same amount of blood enters the heart at the same time during diastole. Any discrepancy in the time or in the quantitative relations may ultimately cause heart failure.

SPECIAL JUNCTIONAL TISSUES OF THE HEART

Cardiac muscle consists essentially of certain specialised structures which are responsible for initiation and transmission of cardiac impulses at a higher rate than the rest of the muscle. Those specialised cardiac tissues operate such mechanism are collectively known as the junctional tissues of heart. They comprise the following structures : (1) Sino-atrial (S. A.) node. (2) Atrioventricular (A. V.) node. (3) Bundle of His (atrioventricular bundle). (4) The right and left branches of the bundle - ending in the (5) Purkinje fibres.

The sino-atrial and atrioventricular nodes and bundle of His are composed of specialised cardiac tissue and contain high amount of glycogen. These have got more sarcoplasm than

Contd...P/4

the rest of the cardiac muscle fibres. Purkinje fibres also contain high amount of glycogen in their sarcoplasm. The atrial muscle fibre is connected with the ventricular muscle fibre only through the bundle of His because a fibrous tissue ring keeps the atrial muscle separated from the ventricular muscle. Damage of bundle of His causes dissociation of atrial and ventricular rhythm.

1. <u>SINO-ATRIAL NODE</u>. (Keith and Flack, 1907). It is situated in the right atrium at the junction of superior vena cava and the right auricular appendage. It extends downwards along the sulcus terminalis for about 2 cm (three-fourths of an inch). It is broader at the top and tapering below, and measures about 5 x 20 mm.

This nodal tissue possesses relatively few myofibrils and also it is claimed to consist of a dense network of small Purkinje fibres. Some investigators believe that the transmission of cardiac impulse from the S. A. node to the A. V. node is mostly facilitated by the presence of Purkinje fibres in the atria. No pathways of special fibres have yet been satisfactorily demonstrated until recently in the walls of the atria.

Goldman (1970) has described that there are three internodal atrial pathways originating from the S. A. node go to the A. V. nodal region (FIG. 1). These internodal tracts contain Purkinje type of fibres. The anterior internodal tract after coming out from the S. A. node curves round the superior vena cava and anterior wall of the right atrium. Here it bifurcates into two branches, one of which goes to the left atrium and other goes to the anterior superior region of the A. V. node. The middle internodal tract and posterior internodal tract after coming out from the S. A. node curve behind the superior vena cava and end in the superior margin and posterior margin of the A. V. node respectively. In between three internodal tracts there are interconnecting fibres which merge just above the A. V. node and also by-pass this node. In between the muscle cells, many nerve cells are found which act as relay stations for the vagus only. Excitor sympathetic fibres are also

Contd...P/5

found here. Functions. It generates the normal cardiac impulse at the rate of 70 to 80 minute in the adult and acts as the pacemaker of heart and the rhythm originated from this region is generally designated as sinus rhythm.

2. ATRIOVENTRICULAR NODE (Tawara, 1906). It is situated in the right atrium at the posterior part of the interatrial septum close to the opening of the coronary sinus. It measures about 2 x 5 mm. The presence of the atrioventricular node was first identified by Kent in 1892 and afterwards His in 1893 described a band of modified muscle fibres to course from the atrium to the ventricle. Tawara (1906) described in details the presence of this specialised tissue in many species of animals. The cells of the A. V. node are cardiac muscle fibres but have a few myofibrils. It also consists of Purkinje fibres which form a dense network. The node is extended into the common-the atrioventricular bundle (bundle of His). Functions. (a) It receives the impulse originating from the S. A. node and transmits it to the ventricles through the bundle of His. (b) It acts as reserve pacemaker. The rhythm that is originated in the A. V. node is known as nodal rhythm. (c) It also initiates the cardiac impulse, but at a slower rate (40 to 60 per minute). In abnormal conditions, when the S. A. node fails, the A. V. node generates the impulse (nodal rhythm).

3. BUNDLE OF HIS. Course - The main trunk of this bundle is continuous with the A. V. node and passes upwards until it reaches the posterior margin of the membranous part of the interventricular septum and then forwards below it. It measures about 20 mm long.

4. BUNDLE BRANCH. Just above the muscular part of the septum, the bundle divides into right and left branches. The right bundle branch is longer than the left one. The left bundle branch bifurcates into superior and inferior divisions. It pierces the membranous septum, enters the left ventricle and passes along the muscular septum towards the apex. The left branch ends in the Purkinje systems of the ventricular subendocardial tissue (FIG. 2). The right branch passes down the right side of the septum. These branches remain just under the endocardium. They are finally distributed through the

Contd...P/6

terminal arborisations of a special type of cardiac muscle fibres, known as the Purkinje fibres. **Functions**. (a) Conduction. Its normal function is to conduct the atrial impulse into the ventricles. (2) Rhythmicity - When the S. A. and A. V. nodes fail, the bundle can originate cardiac impulse. But the rate is very slow, about 36 per minute.

5. PURKINJE FIBRES. The Purkinje fibres which arise from the branches of the bundle of His, spread from the interventricular septum directly to the papillary muscle and then to the lateral walls of the ventricle ending ultimately within the subendocardial network. Purkinje (1845) first observed the presence of these fibres in the subendocardial tissue of the ungulate, heart. Purkinje fibres have got a larger diameter (50 to 70 p) than the ordinary cardiac muscle fibre (15 p). It also contains relatively more sarcoplasm with large amount of glycogen. Myofibrils in the fibre are present mostly in the periphery of cells and the central space is occupied by glycogen. Functions. Main function of these fibres is to conduct impulse quickly to every part of the ventricular muscle fibre. These fibres also can initiate impulse (30-35 per min.) in case of atrioventricular dissociation.

SPREAD OF CARDIAC IMPULSE
CONDUCTION OVER ATRIAL MUSCLE.

Cardiac impulse originated at the S. A. node is transmitted over both the atria like concentric waves. The spread of electrical impulse through the S. A node is very slow (0.05 m per sec.) but the same through the junctional tissues that connect the node to the atrial musculature or to the A. V. node is higher (1 m per sec.).

CONDUCTION OVER A. V. NODE:

There is also a considerable delay of 0.07 sec. to 0.1 sec. in transmission of impulse in the A. V. node before excitation spreads over the ventricle. This A. V. nodal delay allows the atrial systole to complete before the ventricle is excited. This delay is observed maximally at the junctional region between the atrium and atrioventricular node. The conduction velocity of impulse at this region is approximately 0.05 m per sec.

Contd...P/7

CONDUCTION OVER BUNDLE OF HIS AND THE RIGHT AND LEFT BUNDLE BRANCHES.

Beyond the atrioventricular region, the impulse is transmitted along the bundle branch at a higher velocity (4-5 m per sec.). The impulse from the bundle of His passes quickly through the right and left bundle branches and ultimately reaches the Purkinje fibres and ventricular muscle fibres as well.

CONDUCTION THROUGH PURKINJE SYSTEMS.

The impulse, after passing through the right and left bundle branches, passes into the Purkinje fibres and also its multiple ramifications within the subendocardial surfaces of both ventricles. The impulse then travels from the endocardium to the epicardium of ventricular muscle perpendicularly.

CONDUCTION THROUGH VENTRICULAR MUSCLE.

In human beings, the midportion of the interventricular septum is activated normally in a left to right direction. So the depolarisation of the ventricular muscle begins at the left side of the interventricular septum because the Purkinje fibres arise more proximally from the left bundle branch than from the right bundle branch and activates the left side of the septum initially. After midseptal activation from the left to the right direction (FIG. 3), the impulse comes down the septum to the apex of the heart and next portions of myocardium that is activated is the anteroseptal region of the ventricular myocardium (FIG. 4). The impulse then proceeds along the right and left ventricular walls to the atrioventricular groove. The spread of excitation through the ventricle proceeds from the endocardium to the epicardium and thus the whole of the right and left ventricular walls are depolarised (FIG. 5). The portions of the ventricles that are excited lastly are the posterobasal regions of the left ventricle, the pulmonary conus, and the uppermost portion of the interventricular septum (FIG. 6).

CELL-TO-CELL CONDUCTION.

Earlier conception was that there is protoplasmic continuity between cells of the cardiac muscle and thus impulse

Contd...P/8

is transmitted through the intercellular bridges. But electron microscopic studies reveal that there is no such bridges or protoplasmic continuity, the cells are bounded on all sides by membranes of high resistance. Transmission of impulse through this membrane is impossible. But the intercalated disc which crosses the short axes of the cells offers very low resistance and impulses reaching the intercalated disc are quickly propagated to the cells. So the syncitium-like properties of cardiac muscle are due to presence of low resistance intercalated discs (FIG. 7).

INTRACELLULAR CONDUCTION.

Through the specialised areas of the intercalated discs, the impulse ultimately reaches the cell membrane - sarcolemma. From here, the impulse is transmitted quickly through the transverse tubules (T tubules) - sarcolemmal invagination. This T tubule passes through the Z-line and here it is in close apposition with the sarcoplasmic reticulum that is present in the area in between the Z-lines. So the impulse from the cell wall is transmitted through the transverse tubules and then through the sarcoplasmic reticulum, and reaches ultimately to the contractile units of the muscle (FIG. 7).

BASIC PROPERTIES OF CARDIAC MUSCLE

The properties present in other muscles are also shown by the cardiac muscle. But it shows certain special features. They are briefly summarised below:

1. RHYTHMICITY.

One of the main characteristic features of the cardiac muscle is that it can initiate its own impulse rhythmically. This inherent rhythmical property is present throughout the cardiac muscle as evident from the electrophysiological studies of the single fibre from the S.A. node, A. V. node, atrial muscle, Purkinje fibre and also from the ventricular muscle fibre. If a strip of muscle fibre from the atrium or ventricle is perfused in normal physiological solution with proper ionic concentrations, pH, temperature, etc., then it beats

Contd...P/9

rhythmically, proving the presence of pacemaker activity. It has been discussed earlier that the rate of rhythmicity in the S. A. node is 70 to 80 per minute, in A. V. node 40 to 60 per minute, in atrium 60 per minute, in ventricle 20 to 40 per minute. Due to higher rhythmical property of the S. A. node, it controls the rest of cardiac muscle and thus heart beats at the rhythm of the S. A. node. When the S. A. node fails, the A. V. node takes the charge and if it fails, the atrium and afterwards ventricle take the charge of maintaining heart beat.

Transmembrane potentials, recorded simultaneously from the single fibre of the S. A. node, A. V. node and ventricle during normal beating, vary in their successive phases from those are recorded separately in isolated preparation. Difference in membrane potential recorded in between outside and inside cell membrane is known as transmembrane potential. Transmembrane potential can be recorded by inserting micro-electrode (0.2 p) directly into the single cell and the indifferent electrode is kept outside the cell. As soon as the micro-electrode penetrates the cell membrane and if the indifferent electrode is kept outside the cell, a potential difference ranging from - 80 to - 90mV will be shown in the galvanometer. The resting transmembrane potential, thus recorded in the S. A. node is -80 mV, but the same, in the A. V. node, atrial muscle and ventricular muscle is -90 mV. This resting membrane potential will be maintained until the resting state is disturbed by propagated impulse. With the onset of excitation, the steady state is changed rapidly and the membrane potential attains to + 20 mV. After reaching at this level, it maintains steady state for a while and gets down gradually to its initial resting state. The upward deflection is the depolarisation phase as the membrane permeability to Na^+ is altered due to excitation. In resting state the extracellular Na^+ concentration is higher than that of the intracellular and the permeability of Na^+ to the cell membrane is hastened during excitation. So during depolarisation, there is an influx of Na^+ and this process is maintained until it reaches + 20 mV. Here Na^+ entry is decreased and K^+ begins to come out from the cell. This phase is known as repolarisatiom. Heart remains in a state of systole at this stage. K^+ efflux

Contd...P/10

decreases after reaching the resting level and reorientation of ions (K^+ and Na^+) takes place at this stage by 'pumping', against electrochemical gradient and by metabolic energy. This phase is known as slow diastolic depolarisation. This phase coincides with the period of diastole. This process is very slow and due to reorientation of ions, the threshold potential (-60 mV) is slowly achieved and the membrane is further depolarised and the process is repeated. This process once started is maintained in the cardiac muscle until death ensues. How this process is initially being started in embryonic cardiac tissue is not yet fully known but it is presumed that certain hypoxic state may have been the cause of excitability in the embryonic stage. This slow diastolic depolarisation phase is the characteristic of pacemaker activity. This slow diastolic depolarisation phase of transmembrane potentials is absent in other than pacemaker area if the same is recorded simultaneously from single cell in a pacemaker-dominated heart. This phase is however present in a transmembrane potential recorded from any isolated preparation of cardiac muscle.

Cardiac rhythm is altered following stimulation of vagi or sympathetic nerve supplying the heart. The cause of the slowered heart rate following vagal stimulation is presumably due to prolongation of the slow diastolic depolarisation phase and also due to hyperpolarisation for increased permeability of K^+ to the cells. The rate of firing is decreased and longer time interval is required to achieve the level of threshold potential. Strong vagal stimulation causes the complete disappearance of spontaneous discharge for some time. These effects are in the vagal nerve endings due to liberation of acetylcholine, which causes hyperpolarisation of the cell membrane by increasing the K^+ permeability. On the other hand, cardiac sympathetic nerve stimulation or administration of adrenaline induces the membrane potential to fall more rapidly and the rate of spontaneous discharge increases greatly.

2. CONDUCTIVITY.

Conduction of impulse through different parts of the heart has already been discussed in detail. The impulse

Contd...P/11

originated at the S. A. node spreads over the atria and reaches the A. V. node through the internodal fibres. There is no special connecting tissue between the two nodes, demonstrated histologically but from electrophysiological studies, the existence of such tissue has been reported by Carvalho and others (1961). The A. V. node transmits the impulse through the bundle of His and its branches to the ventricles. From the apex of the heart through the Purkinje fibres the impulse is conducted to the base. Conduction in the bundle of His and the Purkinje fibres is 1 metre per second, still less in the ventricular muscles 0.4 metre per second and least in the S. A. node 0.05 metre per second and A. V. node 0.1 metre per second.

3. EXCITABILITY AND CONTRACTILITY.

Like other muscles, the cardiac muscle is excitable by adequate stimuli and responds by contraction. The fundamental contractile unit of the cardiac muscle is myofibril which contains the protein units, action and myosin. During contraction these two units are associated in presence of ATP and thus the fibre is shortened, but during rest these are dissociated again with the resynthesis of ATP. Myosin itself is an enzyme 'ATP-ase' capable of dephosphorylation of ATP. Ca^{++} ion activates the ATP-ase activity - favouring prompt association of actomyosin and ADP complex. Excess calcium always keeps the muscle unit in contracting state (Calcium rigor) due to association of more contractile units. K^{+} ions do not favour association of actin and myosin (Szent-Gyorgyi and Hajdu, 1952). So if excess K^{+} is added in the extracellular fluid then the heart muscle gradually stops in diastole.

4. ALL-OR-NONE RESPONSE

If a quiescent heart muscle is stimulated at widely spaced electrical shocks of increasing strength then muscle contracts as a whole only when the threshold strength is reached. But there was no such increasing amplitude of contraction with increasing intensities of stimulation. This was observed by Bowditch (1871). Single skeletal muscle fibre behaves like this but if the entire muscle is stimulated with graded intensities of stimuli then graded responses are encountered.

Contd...P/12

5. REFRACTORY PERIOD.

This is another characteristic property of the heart muscle. The refractory period of the heart is long and can be divided into three parts : (1) Absolute refractory period - This period extends throughout the whole period of contraction. Any stimulus, however strong, will fail to elicit a response if it falls within this period. For this reason, heart muscle cannot be tetanised. This long refractory period ensures enough time for recovery of the cardiac muscle. This is the reason why cardiac muscle cannot be fatigued. This period coincides the period from the onset of depolarisation phase to the repolarisation up to the threshold potential (FIG. 170).
(2) Relative refractory period - This starts immediately after the absolute refractory period and involves the first part of relaxation. Only a very strong stimulus will be effective. This period begins when the transmembrane potential during repolarisation phase has just reached the threshold potential (-60 mV) and ends just before the repolarisation phase is ceased.
(3) There is another type of refractory period observed after the relative refractory period which is known as supernormal period. This period is limited from the point of termination of repolarisation to the beginning of slow diastolic repolarisation phase.

6. TONE.

Heart muscle possesses tone. This tone is independent of nerves and can be adjusted. In this way, it can maintain a fairly constant tension upon its varying contents.

CARDIAC CYCLE

DEFINITION

Changes that occur in the heart during one beat, are repeated in the same order in the next beat. This cyclical repetition of the various changes in heart, from beat to beat, is called cardiac cycle.

Contd...P/13

At the beginning of ventricular diastole, the semilunar valves close produring the second sound. There is a brief interval between the beginning of diastole and the closure of the semilunar valves - known as the protodiastolic period (0.04 sec.). So that, second sound occurs actually after this period. The A. V. valves open a little after the closing of the semilunar valves. The interval between these two is called the isometric relaxation period (0.08 sec.). During this period ventricles relax as closed cavities and intraventricular pressure steeply falls. At the end of this period, the intraventricular pressure goes below that of the atria and the A. V. valves open. Atrial blood rushes into the ventricles. producing the third sound. Here, ventricular filling begins. The first part of filling is very rapid, being known as the first rapid filling phase (0.113 sec.). The maximum filling takes place during this brief period. The intermediate part of filling is very slow and is known as diastasis or slow inflow phase. Although this is the longest phase (0.167 sec.), yet the amount of filling is minimum. The last part of diastole corresponds with atrial systole. Due to active contraction of the atria, filling becomes very rapid. This last rapid filling phase (0.1 sec.) is responsible for the last part of ventricular filling. Due to rapid rush of blood, another sound is produced - the so - called fourth sound of heart. Here, ventricular diastole ends and systole commences again. In this way the cycle continues (FIG. 8).

- 0 -

NERVE CONDUCTION

by Dr. Jayanta Kumar Panda

The importance of nervous system lies in the fact that it controls and integrates the different bodily functions, and acts as the chief coordinating agency in the animal body. The nerve tissue is differentiated into two lines (i) neural cells - neuroblasts, and (ii) glial cells - spongioblasts. The glial cells or neuroglia perform as interstitial tissue in grey and white matter whose main function is support, insulation and phagocytosis, whereas the neuroblasts pass through successive stages and ultimately give rise to the matured neurone.

Neurone :

A nerve cell with all its processes is called a neurone (Fig. 1). It is the structural and functional unit of nervour system. It may consist of a nerve cell body or soma and two types of processes - axon and dendrite. Dendrites branch extensively close to the cell body and carry excitation to the cell body, whereas axons spread excitation away from the cell body. The structures contained in the cell body viz. (i) nucleus, (ii) neuroplasm with neurofibrils passing through the neuroplasm from dendrite to the axon, and nissl bodies (granules), (iii) mitochondria, (iv) golgi apparatus, (v) ribosome, (vi) endoplasmic reticulum, (vii) centosome, (viii) cell inclusions and (ix) neurosecretory materials.

PHYSIOLOGY OF NERVE CONDUCTION

Resting potential :

In a state of rest there is a difference in potential of the order of 60-90 millivolts between the outer surface of a cell and its protoplasm, the cell surface being electrically positine with respect to the protoplasm.

Origin of resting potential :

According to Hodgkin and Huxley (1952) bioelectrical potentials are caused by inequal concentration of pottassium,

Contd...P/2

sodium and chlorine ions within the cell and outside it and by the variable permiability of the surface of the membrome to them.

The protoplasm of nerve and muscle cells contains 30-50 times as many pottasium ions, 8-10 times fewer sodium and 50 times fewer chlorine ions as does the extra To the structural elements of the membrome are bound various which lend a particular electric charge to the walls of its there by impeding or facilitating the passage of other ions. is supposed for example, that the presence of dissociated phosphate and carboxyl groups is the reason only the membrome nerve fibre is much less permiable to anions than to cations. Permiability to different cations also various and charges in the different functional conditions of the tissue. At rest the permiability of nerve fibre membrome to pottasium ion is between 20 to 100 times that to sodium ions, where as in an state the ratio is significantly reversed.

***ACTION POTENTIAL :**

If a sufficiently strong stimulus (for instance electric shock) is applied to part of a nerve fibre, it will give rise excitation, the main manifestation of which is a rapid variation of the membrome potential, which is known as action potential.

* (During the resting state, the potassium ions, due to their higher concentration, tend to diffuse out ward and this creates an internal negativity. However, the internal negativity becomes great enough to prevent further movement of pottasium and thus an equillibrium is reached. This is the resting potential A cross the resting plasma membrome the resting potential is usually about 60 to 70 mV .)

1. EXCITABILITY. The nerve fibre can be stimulated by a suitable stimulus, which may be mechanical, thermal, chemical or electrical. In experiments, electrical stimulation is usually employed because its strength and frequency can be accurately controlled. The following changes will show that a nerve has been excited: (a) The muscle or the gland where the nerve ends will

Contd...p/3

espond. (b) The stimulated spot on the nerve becomes electri-ally negative and this wave of negative potential passes along he nerve and can be detected by galvanometer or by CRO. (c) The ction potentials - An electrical disturbance always accompanies he travelling nerve impulse. In resting cell the surface is ositively charged and the interior is negatively charged. When he surface is stimulated and the permeability is increased as result there is reversal of polarisation. The surface at the timulated point becomes negative (cathode) causing catelectro-onic change. When this change rises to threshold level, impulse ill pass like selfpropagated disturbance by drawing positively harged particles from the neighbouring points which in turn ecomes cathode. The depolarisation of the membrane is the first tep of the manifestation of an impulse. After an initial slow ise, depolarisation wave overshoots rapidly and reaches the sopotential line (zero line) to approximately + 35 mV. After hat it reverses and begins to fall very rapidly towards the esting level (- 70 mV). At approximately two-thirds of epolarisation, the rate of fall is being abruptly slowed. This lower fall is known as negative after-potential (after-epolarisation). The rapid rise of depolarisation wave and the apid fall of repolarisation wave are known as spike potential. fter reaching the basal level the wave overshoots slightly ut slowly in the hyperpolarising direction. This is known as ositive after-potential (after-hyperpolarisation). The whole equence of potential changes in the nerve following excitation s known as action potential or membrane potential (FIG. 2).

ECHANISM OF THE DEVELOPMENT OF ACTION POTENTIAL

In resting state the nerve fibre remains in polarised tate and the membrane potential lies within - 70 mV. The inside f the nerve is negative and the outside of the nerve is positive. Na^+ concentration outside the membrane is higher than that of nside the membrane. K^+ concentration inside the membrane is lso higher than that of outside the membrane. K^+ can permeate hrough the membrane at resting state but the Na^+ cannot permeate. rmeability of Na^+ to membrane is increased only after excitation

Contd...P/4.

and it is the first event of the action potential. The action potential occurs in successive stages of depolarisation, repolarisation, negative after-potential and positive after-potential. It has been postulated that in resting state calcium ions (Ca^{++}) remain binding to the protein surfaces of the membrane pores and it does not allow Na^+ to permeate through these resting pores. During excitation Ca^{++} is dislodged from its binding site and the permeability to Na^+ is increased. So the depolarisation starts with the onset of Na^+ entry and thus an increase in Na^+ conductance is taken place. The tremendous increase is Na conductance during this period is known as activation of membrane. Due to this, the reversal of potential is caused with the development of positivity inside the membrane and negativity outside (FIG. 3). But with the increase of positivity inside, further entry of Na^+ is prevented and calcium begins to bind with the proteins of the membrane pores. But as soon as the action potential attains the voltage approximately + 35 mV, K^+ begins to come out from inside the membrane. The inside becomes negative and outside becomes positive again (FIG. 4). This stage is the repolarisation phase and K^+ conductance is increased to the maximum. The mechanism underlying the process of K^+ conductance is mostly hypothetical and increased positivity inside the membrane due to Na^+ entry during depolarisation phase, allows the K^+ to come out and the resting potential is slowly achieved. But at the later period of this phase (at the termination of spike potential) K^+ conductance is slowed down and thus a few milliseconds are delayed in restoring the membrane potential. This state is known as negative after-potential which has been described to be the cause of increased K^+ concentration outside the membrane. This increased K^+ concentration may hinder further efflux of K^+. With the disappearance of the negative after-potential, though the resting membrane potential is achieved yet the resting ionic status is not established. It is achieved by the active Na^+ pump mechanism and Na^+ begins to come out from inside the membrane creating negativity again (Figs. 5). The positive after-potential is due to this process of Na diffusion from inside to outside the membrane. The negativity, produced due to active Na^+ pump mechanism, causes the K^+ to diffuse back to the interior of the nerve fibre. For the active Na^+ and K^+ pump mechanism high energy phosphate (ATP) is required. In this way resting normal ionic status is established during the period of positive after-potential.

Contd...P/5

Excitablity depnds upon the following factors :
(i) strength of stimulus - A minimum strength is essential.
(ii) Duration of stimulus - The stimulus must continue for a certain minimum period, which varies inversely as the strength. Excitability of a nerve fibre can be determined by studying its strength-duration relationship (threshold stimulus intensity duration) of the stimulus. Current intensity of stimulus which is just adequate to cause an impuse is called the threshold. Intensity below the threshold is known as subliminal. Magnitude of current just sufficient to excite a nerve or muscle is called rheobase and the minimum time required to have a response is known as utilisation time. The shortest duration of current flow which will excite the nerve or musculle under current strength equal to twice the rheobase is called the chronaxie. Chronaxie value is a useful index of the relative excitability of the tissues. (Fig. 6)

2. <u>CONDUCTIVITY</u> : The nerve impulse is conducted along the nerve fibre. Conductivity shows the following characteristics: (i) Impulse is propagated along a nerve in both directions. (But under normal conditions the nerve impulse travels in one direction only - in the motor nerve towards the responding organ ; in the sensory nerve towards the centre. This is due to the action of 'synapse'.) (ii) Velocity of nerve impulse - The nerve impulse is propagated with a definite speed (other conditions remaining same). The conduction velocity depends upon the diameter of the nerve fibres, the thicker fibres showing higher velocity. The conduction velocity also depends upon the presence or absence of myelination and also on temperature.

<u>FACTORS AFFECTING CONDUCTIVITY AND EXCITABILITY:</u>

(a) Temperature - cooling diminishes and warming increases these properties. (b) Mechanical pressure-depresses conductivity and excitability. (c) Blood supply - CO_2 and narcotics, viz., ether, chloroform, alcohol, novocain, etc., diminish and finally abolish excitability and conductivity. (e) H-ion concentration - increased pH (alkali) increases and decreased pH (acid) diminishes. At pH 8.0 the nerve becomes

Contd....P/6.

hyperexcitable and spontaneous discharge may occur. Even a single stimulus may cause multiple response (repetitive response (f) Effect of ions - lack and excess of Ca have the same effects as rise and fall of pH respectively. Changes of K^+ exert opposite effects. Na^+ and Mg^{++} - similar to K^+ but less in degree (hence, Na^+, K^+ and Mg^{++} - are neuro-excitatory, while Ca^{++} is neurosedative). (g) O_2 lack-depresses and if continued abolishes these properties. If O is readmitted, they return.

3. ALL-ON-NONE LAW : If the stimulus be adequate a single nerve will always give a maximum response. If the strength or duration of the stimulus be further increased no alteration in the response will take place. This property is present in single fibre preparation. In the whole nerve this property is different.

4. REFRACTORY PERIOD : When the nerve fibre is once excited, it will not respond to a second stimulus for a brief period. This period is called absolute refractory period. The absolute refractory period means that the nerve is completely refractory to stimulation-in other words is incapable of eliciting an action potential at any intensity of stimulation. During the absolute refractory period there is total inactivation of the sodium carrier mechanism and as the Na ions cannot enter the fibre, there is no development of the action potential. Immediately following this, there is a brief relative refractory period, during which the excitability is subnormal but gradually rising. This is succeeded by a third brief period of increased excitability, known as supernormal phase. Lastly, there is a period of subnormal excitability-subnormal phase.

5. SUMMATION : (Latent addition). In a nerve fibre summation of two submaximal stimuli is possible.

6. ADAPTATION : The nerve fibre quickly adapts itself. Due to this adaptation there is no excitation during the passage of a constant current. Only when the strength of the current is suddenly altered or the current is made or broken excitation takes place. A gradual change will fail to excite.

Contd...P/7.

7. ACCOMMODATION : If a stimulus even with stronger strength is applied very slowly to a nerve, then there may have no response only due to lack of attaining the threshold strength. This phenomenon is called accommodation, i.e., slowly applied stimulus is accommodated by the nerve no matter how strong the stimulus is applied.

8. INDEFATIGABILITY : In the nerve muscle preparation, if the nerve is stimulated repeatedly, then after a certain period the muscle fails to give any response. Now if that nerve is isolated from the muscle and placed on a fresh muscle, then application of stimulus will excite the muscle. This shows that nerve is not fatigued.

THE FOLLOWING IS A BRIEF SUMMARY :- (i) Unlike muscles, the excitability, conductivity and the recovery process can go on in a nerve for a considerable period even in absence of oxygen. (ii) The chemical changes in the nerve are roughly of the same nature as seen in the muscles. Pyruvic acid is formed and if O_2 supply be insufficient, lactic acid accumulates (same as in muscles). Thiamine which is essential for complete oxidation of these acids, is found in good amount in the nerve fibres. Although carbohydrates burn, yet they are not the only source of energy (contrast with nerve cells-which possibly use galactose). The breakdown of phospholipids also takes an essential part here. It is said that, the energy requirement of the resting nerve is supplied by combustion of sugar and phospholipids mainly. During activity, ATP and creatine phosphate break down and supply energy for the propagation of the nerve impulse. Both ATP and creatine phosphate are then resynthesised but the source of energy of this recovery process is not known (may be in the same way as in muscles). (iii) During activity acetylcholine is liberated by the cholinergic fibres, while norepinephrine by the adrenergic fibres. (iv) The nerve fibres are rich in K^+ and thiamine. During activity K^+ (and possibly thiamine) diffuses out and Na^+ and enters the fibres. This migration of K^+ and Na^+ seems to be intimately related to the properties, viz., excitability, conductivity, etc., of the nerve fibres.

Contd...P/8.

EXCITATION AND THE LOCAL CIRCUIT THEORY

It was suggested long ago that propagation of a nerve impulse depends on the flow of current in local circuits ahead of the active region, which depolarizes the resting membrane, and causes it in turn to become active. In this local, circuit theory, the flow of current from region A to region B (Fig. 7) results in movement of the active region towards the right. In both myelinated and non-myelinated fibres the principle is the same in that the active region triggers the resting region ahead of it by causing an outward flow of electric current.

CORE CONDUCTOR THEORY OF NERVE IMPULSES

If electrodes are applied to the surface of a nerve fibre, electronic potentials develop on the fibre at the electrodes and in their vicinity. The potentials are the greate underneath the electrode and fall off at points farther away. The development of these potentials and their associates is explained by considering the nerve fibre to act as a core conductor; a cylinder of conducting fluid material (axoplasm) with a sheath (cell membrane) of high electrical resistance, surrounded by a layer of conducting medium.

SALTATORY CONDUCTION OF NERVE IMPULSES

Conduction of nerve impulses is very rapid in myelinated nerve fibres in comparison to those without a myelin sheath. The myelin sheaths are interrupted regularly at spaces known as the nodes of Ranvier. At these nodes the membrane of a neuron makes contact with the surrounding fluid. The distance between successive nodes is more in neurons of large diameter than in neurons of small diameter. Conduction in myelinated neurons is supposed to take place by jumping or skipping of the action potentials from one node to another instead of travelling along the nerve membrane. Such skipping movement from node to node is called saltatory conduction (Fig. 7)

SYNAPTIC TRANSMISSION

The synapse, according to Sherrington (1898), is the functional connection between two neurons. Between the pre-

Contd...P/9

synaptic and post-synaptic membranes a cleft of several angstrom units is present.

ELECTRICAL THEORY OF SYNAPTIC TRANSMISSION

In the crayfish giant motor fibre, it has been demonstrated that impulses pass from the pre-synaptic portion to the post-synaptic membrane. The depolarization here spreads across the synapse from the pre-synaptic portion and is measureable in the post-synaptic membrane. Any depolarization in the post-synaptic fibre does not cause hyper-polarization of the pre-synaptic portion indicating an undirectional flow of current. Thus, the synapse acts as a rectifier with a high resistance towards flow of current in the opposite direction. This type of conduction may also take place in other animals.

CLASSIFICATION OF SYNAPES

On the basis of the nature of connections between neurons, synapses have been classified into three types.

AXOSOMATIC SYNAPSES. In this type, the terminal processes of the presynaptic neuron end on the cell body or soma of the post-synaptic neuron. This type of synapses are found in the cerebellum between the basket cells and Purkinje cells.

AXO-DENDRITIC SYNAPSES. The terminal processes of the axon of the presynaptic neuron end in the dendrites of the post-synaptic neuron on (Fig.8). In the cerebellum, the climbing fibres from connections with dendrites of the Purkinje cells.

AXO-AXONIC SYNAPSES. If the terminal processes of the presynaptic axon make connections with the terminal processes of the post synaptic neuron, this type of synapses are known as the axo-axonic synapses.

STRUCTURE. Electronmicroscopic studies have revealed that the axons of the presynaptic neurons end in expanded terminals-the synaptic or terminal knobs or buttons. The membrane of the synaptic knob is the presynaptic membrane while that of the cell body is the postsynaptic or subsynaptic membrane. Though there is intimate contact between the two membranes, they are separated by a gap, the synaptic cleft

Contd...P/10

measuring about 10-20 nm. Thus, there is no physical continuity in the cytoplasm of the presynaptic, and postsynaptic neurons at the synapses. However, at some synapses, the two membranes show areas of closer proximity and the outer layers of the unit membranes of the pre and post synaptic membranes even fuse, forming gap junctions found in the spinal neurons of teleost fishes and giant fibers of cray fish. In brain cortex parallel intersynaptic filaments (Fig. 9) extending between the pre and subsynaptic membrane in the synaptic cleft have been demonstrated by D. Roberits (1961). The function of the filaments is not yet known. In same synapses (brain cortex and retina), the filaments form a web like network on the subsynaptic membrane extending even into the Cytoplasm of the postsynaptic neuron. This network is known as the subsynaptic web. The Cytoplasm of the terminal knobs contains mitochondria and synaptic vesicles. The synaptic vesicles are spherical or ovoid shaped structures with diameters ranging between 20-65nm. The vesicles are bound by a 4-5 nm thick unit membrane. The vesicles are more concentrated towards the synaptic cleft. The synaptic vesicles contain the excitatory neuro-transmitter substances that mediate transmission of nerve impulses from the presynaptic to postsynaptic neurons. The vesicles may be found on both sides of the synaptic junction, and in electrically transmitting neurons also.

CHEMICAL THEORY OF SYNAPTIC TRANSMISSION

The transfer of a nerve impulse across a synaptic junction is known as synaptic transmission. This process is believed to prought about by the release of chemical substances at the synapse and takes place in the following steps -

1. RELEASE OF THE NEUROTRANSMITTER SUBSTANCE. The neurotransmitter substances synthesized in the terminal processes of the axons are stored in the synaptic vesicles. On arrival of an nerve action potential through the axon into the terminal knobs, by some unknown mechanism, the vesicles release the neurotransmitter substance. Calcium ions are required for the release, and magnesium inhibts the process. After the release of the transmitter the vesicular membrane moves into the cell cytoplasm and is used to package new transmitter substance synthesized.

Contd...p/11

2. DIFFUSION OF THE TRANSMITTER SUBSTANCE. The neuro transmitter substances released by the presynaptic terminal diffuse across the synaptic cleft and bond to specific receptor sites on the subsynaptic membrane. In the vertebrate neuromuscular junction acetyl choline is released from 100-300 presynaptic sites and diffuses across a distance of less than 1 p m.

3. PERMEABILITY ALTERATIONS IN THE SUBSYNAPTIC MEMBRANE. The binding of the neurotransmitter to the receptor molecules is accompanied by alterations in the permeability of the subsynaptic membrane. Two types of alterations in the permeability are observed. The first is a general type in which the permeability of the postsynaptic membrane to all types of ions bringing about a depolarization of the membrane and excitatory post synaptic potential is produced. The second type increases the permeability of the membrane to K^+ and chloride ions causing hyperpolarization of the membrane and inhibitory post synaptic potential. If the synaptic potentials are great enough to produce sufficiently strong local currents, a spike is generated in the appropriate region of post synaptic neuron.

4. DESTRUCTION OF THE NEUROTRANSMITTER SUBSTANCE. The neurotransmitter substance is destroyed quickly so that normal subsynaptic resting potentials are restored and the neuron may respond again to a new stimulus.

CHEMICAL NATURE OF TRANSMITTER SUBSTANCES. Acetylcholine has been considered to be the transmitter substances of the (1) cholinergic effector organs, (2) postganglionic autonomic nerve endings (3) preganglionic sympathetic nerve endings, (4) at some sites of the CNS, (5) neuromuscular junction.

NATURE OF TRANSMISSION IN THE CNS

CHOLINERGIC TRANSMISSION. Role of acetylcholine (ACh) in the transmission of nerve impulse in the cholinoceptive synapses at the neuromuscular junction, autonomic ganglia, etc., is well known (Fig. 10). The presence of ACh in the CNS has been known for last four decades. Being distributed non-homogeneously it

Contd...P/12.

performs a specific function in some region but not in all regions. Little ACh is present in the dorsal root, the optic nerves, and the crebellum. Brain stem, thalamic nuclei and cerebral cortex contain moderate amount, but caudate nucleus and the retina contain very large amount. Nature of distributio of true acetylcholinesterase (AChE) is roughly parallel to that of ACh. Studies with the multiplebarrelled micropipette it is observed that transmission in the recurrent axon synapses upon Renshaw cells is cholinergic in nature and it resembles those in neuromuscular junction and in postganglionic cells in the autonomic ganglia. With studies in central neurones, it is observed that 10% of cortical neurones are activated by cholinergic synapses. Pseudocholinesterase is also present in various parts of the CNS.

CENTRAL ADRENERGIC TRANSMISSION. Norepinephrine is the major transmitter agent in the postganglionic sympathetic neurones. Its presence in the ganglia can be demonstrated by histofluorescent methods.

The presence of adrenergic substances in the extracts of the mammalian brain and also its regional distribution in the brain have been described as early as 1954 and indicated that adrenergic transmission is possible. Whole brain concentrations of catecholamines are about 0.1 to 0.5 pgm per gm. Midbrain, pons, medulla, cerebral cortex, hippocampus, cerebellum and spinal cord contain catecholamines in lower concentration. In the hypothalamus, olfactory bulb, retina, median eminenca, limbic system, large amounts of amines are present. In the spinal cord, there is a system of descending adrenergic fibres which terminate about neurones of the intermediolateral horn. Central adrenergic systems have also been mapped out by following the technique of fluorescent.

Mechanisms of synthesis, storage, inactivation and removal of norepinephrine are present in the CNS and are in general similar to those in peripheral adrenergically innervated tissues. As catecholamines cannot cross the blood-brain barrier, the enzymes and precursors required for norepinephrine synthesis are present in the brain.

DOPAMINE, the immediate precursor of norepinephrine is also suspected to act as transmitter agent in certain motor function of the CNS.

%%%%%%%%%%%%%%%

PLANT GROWTH HORMONES

Dr. R.Misra
Lecturer in Bdany

A plant hormone is an organic compound synthesized in one part of a plant and translocated to another part where, in every low concentration, it causes a physiological response. The response in the target organ is not necessarily promotive; there are plant hormones that are seen to have inhibitory effects on physiological processes like growth and differentiation. Basing on their action two types of plant growth hormones have been recognised: growth promoters and growth inhibitors. Inorganic ions e.g. K^+ or Ca^{2+} which have well known growth responses are not growth hermones. Neither are synthetic organic compounds like 2,4 - D considered as hormones - such synthetic organic compounds with hormone like effect are known as plant growth regulators. A hormone must essentially be synthesized and translocated within the plant. Hormones are also required in very minute doses usually concentrations of μM or less. There are still only five groups of well accepted hormones. They include Auxein, gibberellins, cytokinins, obscisic acid and ethylene.

THE AUXINS

The term auxin (GK Auxein = to grow) was first used by Frits Went when he observed the curvature of oat (*Avena sativa*) coleoptiles towards light. This curvature phenomenon, called phototrapism, was explained to be the result of a substance present in the coleoptile tip. The substance which was named as 'auxin' could diffuse from cut tips to agar blocks when placed below. The agar block could new replace the effect of the coleoptils tip (Fig.1).

Went's auxin was later identified as indole -3-acetic acid (IAA, Fig.2) Subsequently the word 'auxin' was used as a generic name to describe all compound having effects similar to IAA. 4-chloro-indoleacetic acid (4-chloro IAA) and Phenyl acetic acid (PAA) are auxins widespread among plants. Similar compounds like naphthaneacetic acid (NAA), undole berlhrie acid (IBA), 2,4 - dichloro phenixy cocetic acid (Fig.2) synthesised and having physiological responses common to IAA are also considered to be auxins, but as mentioned above these ar not growth hormones but classified as plant growth regulators.

IAA, one of the common naturally occuring auxin is synthesised in young tissues such as shoot meristems and growing leaves. It is synthesised from the amino acid trypto-phan and is transported in a basipetal direction normally via parenchyma.

Physiological Effects of Auxins:

According to the vast array of literature available regarding auxin , the effect of auxins is stimulatory in some cases and inhibitory in other. Some of the important function are discussed below.

a) Cell elongation: Auxins ~~induce~~ induces cell elongation by in tiving cells especially in the young cells.

b) Phototropism: The differential growth of shoot tips which causes phototropic movement of the shoot is because of unequal distribution of auxins in illuminated and non illuminated side which is probably accomplished by light-reduced inactivation of auxin or transport of auxins from illuminated to the non-illuminated side.

c) Geotropism: If an intact seedling is placed in a horizontal position, it will respond to the earth's gravitational field with a particular pattern of growth. The positively geotropic movement of the root and negatively geotropic movement of the shoot is explained by the transport of auxins away from the gravity in the roots and towards gravity in stems.

d) Root initiation: Application of auxins to cuts ends of stems initiates the differentiation of adventious roots in cuttings - Auxins are also used in tissue -culture media for differentiation of the callus.

a) Parthenocarpy: Exogenous manipulation of auxins induces parthenocarpic development of fruits.

b) Abscission: The presence of auxins prevents the formation of abscission layers in fruits and leaves.

GIBBERELLINS

The gibberellins are another class of compounds whose minute quantities profoundly stimulate the growth of many plants.

DISCOVERY

The gibberellins were discovered in an interesting and incidental way. In early part of the twentieth century, Japanese farmers noted that some plants in rice fields were taller, thinner and paler than the normal plants and were sometimes devoid of fruits too. They named this disease as "bakanae", meaning foolish seedlings. Sawada (1912) suggested that the disease is due to a 'substance' secreted by a parasitic fungus, Fusarium moniliforme (syn. Gibberella

Contd...P/4.

fujikuroi) infecting the diseased plants. This suggestion was experimentally supported by Kurosawa (1926) who demonstrated that sterile filtrates of the fungus could initiate symptoms of bakanae disease in healthy rice seedlings. Later in 1939, Yabuta and Hayashi isolated this growth promoting substance in crystalline form and named it as gibberellin A, which has now been shown as a mixture of many growth promoters collectively known as gibberellins.

Since that time, gibberellins and allied substances have been found in higher plants also by Mitchell et al. (1951), West and Phinney (1957) and Sumiki and Kawarada (1961).

DEFINITION

A gibberellin (abbreviated as GA) may be defined as a compound which is active in gibberellin bioassays and possesses a gibbane ring skeleton (refer page 495). There are, however, other compounds (like kaurene) which are active in some of the assays but do not possess a gibbane ring. Such compounds have been called gibberellin-like rather than gibberellins.

ISOLATION, DISTRIBUTION AND BIOASSAY

About 29 gibberellins have been isolated so far and their chemical structures known. These have been named as gibberellin A_1 (GA_1), gibberellin A_2 (GA_2) and so on up to gibberellin A_{29} (GA_{29}). Of these Cross et al (1961) have isolated 6 gibberellins from the fungus. Fusarium moniliforme and designated them as GA_1, GA_2, GA_3, GA_4, GA_7 and GA_9. The same year, MacMillan et al isolated 3 gibberellins from bean seeds and named them as GA_5, GA_6 and GA_8. The GA_{10} and GA_{13} have been discovered by Mulholland (1963). All these compounds are sometimes referred to as constituting the gibberellin A series.

Contd...P/5.

Although the gibberellins wer originally isolated from a fungus, but now they have been shown to be present in almost all the groups of plant kingdom. For example, GA_1 and GA_5 have been isolated from immature seeds of Phaseolus vulgaris by West and Phinney (1959). Although all the organs of the flowering plants contain gibberellins, but the highest level has been detected in seeds. Young leaves and roots are also rich in them. It may, thus, be generalized that rapidly growing and developing regions of the plant possess higher concentrations of gibberellins.

The gibberellins can exist in more than one form within the plant. Hashimoto and Rappaport (1966) suggested that the esterified forms of gibberellins (i.e., neutral gibberellins) act as reservoir of active gibberellins. The active acidic form may be drawn from the neutral form as and when needed. In addition, bound forms of gibberellins also exist (mr Comb, 1961).

Many angiospermous plants have now been used as bioassay for gibberellins and gibberellin-like substances. A few of them are Avena sativa (lead section), Pisum sativum (intact seedling), Triticum vulgare (excised coleoptile) and Rudbeckia bicola (rosetted plants).

CHEMISTRY

The chemical structure of the gibberellins was established by Cross et al in 1961. They showed that the gibberellins (Fig. [illegible]) are a group of closely related compounds and possess a common feature, the gibbane ring skeleton. The gibbane ring consists of a carbon skeleton with 4 rings, designated as A, B, C and D. They are hence described

Contd...P/6.

as tetracarbocyclic compounds. Of the 29 gibberellins, 19 are C_{19} compounds and the other 10 have 20 carbon atoms each. Eighteen gibberellins are monocarboxylic, 7 are dicarboxylic and 4 are tricarboxylic acids. The various gibberellins differe from each other in the number and position of the functional groups present in the molecule. In gibberellin A_1, the functional groups are a carboxyl, one ethylenic double bond, two alcoholic hydroxyl groups (one secondary and the other tertiary), a saturated lactone and one methyl group. Gibberellin A_3 differs from GA_1 in the presence of one more ethylenic double bond in the ring A. It is, thus, more unsaturated than GA .

The gibberellin A_2 and gibberellin A_4 both have structures similar to that of GA_1 except for the difference in position of the tertiary hydroxyl group and the absence of a double bond in GA_2 and in the absence of tertiary hydroxyl group in GA_4. The gibberellin A_5 is, in fact, a dehydrogibberellin A_1 where the secondary hydroxyl group is eliminated from ring A, making the compound more unsaturated.

Gibberellin A_3 has been usually shown to be biologically most active followed by GA_1, GA_4 and GA_2 in descending order of their activity.



$$GA_3 > GA_1 > GA_4 > GA_2$$

PHYSIOLOGICAL ROLES

Gibberellins may be regarded as natural phytohormones on account of their wide range of distribution in plants and specificity of response of individual flowering plants to the exogenously applied gibberellins. The gibberellins, however, play important roles in the following processes :

Contd...P/7.

1. GENETIC DWARFISM. In certain plants, dwarfism is caused by the mutation of a single gene. Such individuals are called 'single gene dwarfs'. In these plants, dwarfism is due to shortening of internodes rather than a decrease in the number of internodes. Application of gibberellins on such dwarfs causes them to elongate so much as to become indistinguishable from the tall normal plants. Elongation of the stem, in fact, takes place due to an elongation in the internodes rathern than an increase in the number of internodes. Thus, genetic dwarfism has been successfully overcome by gibberellin A_3 treatment in many single gene dwarf mutants like *Pisum sativum*, *Vicia faba* and *Phaseolus multiflorus* (Brian and Hemming, 1955).

Two views have been put forward regarding the mechanism of control of dwarfism by gibberellins.

(a) It is due to the lack of endogenous gibberellins in dwarf plants or if at all present, they are in traces as to have no effect.

(b) A natural inhibitor is present in those plants which retard growth. And the gibberellin, when applied, nullifies the effect of this inhibitor.

2. BOLTING AND FLOWERING. 'Rosette plants' are characterized by their profuse leaf development and retarded internodal growth. But prior to the reproductive phase, their occurs striking elongation in the internode so that the plant attains 5 to 6 times the original height. Treatment of these 'rosette' plants with gibberellins, under conditions that would normally maintain the rosette form, induces them to bolting (or shoot elongation) and flowering (Lang, 1957). By regulating the amount of gibberellin applied, it is also

Contd...P/8.

possible to separate shoot elongation from flowering; with low dosages of gibberellins, the plant will bolt but not flower (Phinney and West, 1961).

It is, therefore, not amazing to find a direct correlation between the amount of gibberellin present and the habit of the plant, whether rosetted or bolted. Native gibberellin-like substances are found in higher concentrations in the bolted forms than in the nonbolted ones. This has been experimentally demonstrated in quite a few plants including the biennial Hyoscyamus niger by Lang (1957) and the cold-requiring plant Chrysanthemum morifolium and a long-day plant Rudbeckia speciosa by Harada and Nitsh (1959).

As far as the use of gibberellins in agriculture is concerned, it may be possible to grow cold-requiring plants in warm countries and long-day plants in short-day conditions at lower altitudes.

3. LIGHT-INDUCED INHIBITION OF STEM GROWTH. Light-grown plants reveal suppressed stem growth than the dark-grown (or etiolated) plants, indicating that light has an inhibitory effect on stem elongation. But this inhibitory effect of light on dyrm rlongation can be reversed at least in some plants (like Pisum sativum) by the application of gibberellins on these plants. This clearly suggests that endogenous gibberellin is the limiting factor in stem elongation.

Lockhart (1961) has given a possible explanation for it. According to him, exposure to light lowers the level of available gibberellins present in the plant. The lowered available gibberellin contents then, in turn, decrease the plasticity of cell walls, thus inhibiting stem growth. The theory has, however, not won the universal support on account of the following drawbacks :

Contd...P/9.

(a) Stem elongation is also induced in mustard seedlings, grown in dark, upon application of gibberellin.

(b) In some plants, gibberellin-stimulated stem growth has been found to be partially due to enhanced cell division and has nothing to do with cell wal plasticity.

(c) Germination of the seeds of Lactuca sativa is not only promoted by gibberellins but by red light too.

4. PARTHENOCARPY. Like auxins, the gibberellins are also capable of inducing parthenocarpic fruit-set. Gibberellins are, in fact, more efficient than the auxins in inducing parthenocarpy. For example, Wittwer and Bukovac (1957) have found gibberellin to be about 500 times more effective than IAA in inducing parthenocarpy in tomatoes. Moreover, there are cases where auxins have failed to induce parthenocarpy while gibberellins are effective, as shown experimentally for apples (Davison, 1960) and stone fruits (Crane et al. 1960).

Gibberellin-induced parthenocarpy has been reported in many plants such as Cucumis sativus (cucumber), Solanum melongena (brinjal) and Zephyranthes sp. Whether the production of parthenocarpic fruits is a direct action of gibberellins or an interaction with the natural auxins of the plant has not been conclusively proved.

5. BREAKING DORMANCY OF SEEDS. The light-sensitive seeds (lettuce, tobacco) show poor germination in dark and on exposure to light their germination starts vigorously. But when these seeds are treated with GA_3, the light requirement is alleviated and they germinate in dark.

Contd...P/10.

6. BREAKING DORMANCY OF BUDS. In temperate areas, the buds produced in winter remain dormant until the next spring due to very low temperature. The dormancy in such cases is overcome by gibberellin treatment. Thus, GA_3 treatment to birch buds has replaced the light requirement for breaking dormancy (Eagles and Wareing, 1964). Gibberellins are also capable of breaking dormancy in potato tubers.

7. ROLE IN ABSCISSION. GA treatments have shown accelerated rate of abscission in explants of bean (Chatterjee and Leopold, 1964) and of Coleus (Gupta and Kaushik, 1969).

8. STIMULATION OF ENZYME ACTIVITY IN CEREAL ENDOSPERM.

Yomo (1960) and Paleg (1960) working independently showed that the gibberellins applied exogenously could stimulate amylase activity in isolated barley endosperm. It was then shown that it is the aleurone layer of the endosperm which sensitive to the gibberellin. Subsequent researches by Paleg (1964) and Varner (1964) revealed that GA treatment of isolated aleurone can cause release of the enzymes, amylase and proteinase. Finally, Jacobson and Varner (1967) showed that the two enzymes (amylase and proteinase) induced by GA treatment arise through de novo* synthesis. These enzymes participate in the breakdown of the stored starch to simple sugars. These sugars are then translocated to the growing embryo where they provide energy for growth.

9. SEX EXPRESSION. Gibberellins are also capable of altering the sex of the flowers. Galun (1959) could induce maleness by foliar application of GA_3 to the female flowers of Cucumis. Also, the antheridia have been induced to develop in many fern gametophytes by GA_3 treatment.

* A Latin phrase, meaning anew.

Contd...P/11.

RELATIONSHIP BETWEEN AUXINS AND GIBBERELLINS

Auxins and gibberellins are similar to each other in that both promote cell elongation, flowering and parthenocarpy. These, however, differ from each other in many of the physiological activities. These differences are listed in Table 22.2.

Accumulated evidences indicate that auxins and gibberellins act both independently and together, depending upon the type of plant and the conditions under which the plant grows. The fact whether the auxins and the gibberellins interact or not is not conclusively proved.

CYTOKININS

DISCOVERY AND NOMENCLATURE

Auxins and gibberellins, besides inducing cell elongation, also do promote cell division under certain conditions. But this behaviour of them is an exception rather than a rule, However, there exist in plants many substances inducing cell division. For example, Van Overbeek et al (1941) found coconut milk as an active stimulant of cell division. Later, in 1955 Miller et al isolated a "cell-division-stimulating factor" from yeast DNA. It was named as kinetin because of its amazing power to stimulate cell division (cytokinesis) in the presence of an auxin. In subsequent years, many other compounds promoting cell division have been synthesized. Miller and his associates (1956) have grouped all such compounds including kinetin under a generic name kinin. Letham (1963) proposed the term cytokinins for such substances. This term is the most acceptable one.

Fairley and Kilgour (1966), however, prefer to use the term 'phytokinins' for such substances in order to distinguish them from the peptide hormones of animal gastrointestinal tract.

Contd...P/12.

DEFINITION

Skoog, Strong and Miller (1965) have defined cytokinins as chemicals which, regardless of their activities, promote cytokinesis (cell division) in cells of various plant organs.

Fox (1969) has defined cytokinins as chemicals composed of one hydrophilic adenine group of high specificity and one lipophilic group without specificity.

OCCURRENCE

Although kinetin does not occur in nature but other kinins are found occurring widely in plants. The naturally-occurring kinins do not occur free in nature but are normally bound to a pentose sugar, ribose and sometimes to an inorganic phosphate, the ribonucleotide.

Fruits and endosperm are the richest sources of kinins. Coconut milk and corn endosperm possess the active substance. Substances with cytokinin activity have also been reported in tomato juice, in floral extracts of apples and pears and also in cambial tissues of certain plants. A kinetin-kike substance is also present in peach embryo (Powell and Pratt, 1964) and sunflower root exudate (Kende, 1964).

The cytokinins are synthesized mostly in roots and probably originate at the root tips. Whether the shoots also synthesize cytokinins or else receive their cytokinin requirement from the roots is not certain.

CHEMISTRY

Chemically, kinetin ($C_{10}H_9ON_5$) is 6-furfurylamino-purine. It is formed from deoxyadenosine which is a degradation product of DNA (Hall and de Ropp, 1955). The

Contd...P/13.

structural formulae of kinetin and its 3 structural analogues are given in Fig. . All these substances promote cell division.

Apart from the above-mentioned kinins, Letham (1963) successfully isolated a cytokinin in pur crystalline form immature maize seeds. It was named as zeatin (Fig.22.14) and identified as 6-(4-hydroxy 3-methylbut-trans-2-enyl) aminopurine. Zeatin is more powerful than any other known cytokinin probably because of the presence of a highly reactive allylic OH group in its side chain.

Fleissner and Borek (1962) have described compounds such as N^6-methylaminopurine and N^6, N^6-dimethylaminopurine. These are widespread in plants and have cell division stimulating property. Recently, a cytokinin called N^6-purine has been isolated from serine-transfer-RNA of yeast cells by Hall and others in 1966.

PHYSIOLOGICAL ROLES

Certain physiological process which are influenced by the cytokinins esp., kinetin are given below:

1. CELL DIVISION. Kinins are notable for their stimulatory effect on cell division. Using tobacco pith cultures Skoog and Miller (1957) found that, in addition to IAA, kinetin is also needed for growth. The growth response is much more pronounced when both IAA and kinetin are used together in right ratio of concentrations. When either of them is used alone, a little response is produced which is due to the presence of small amounts of endogenous kinetin-like substances and IAA, already present in the tissues.

The process of cell division completes in 3 steps, viz., DNA synthesis, mitosis and cytokinesis. Studying the

Contd...P/14.

specific influence of IAA and kinetin alone on any of these 3 steps, Patau, Das and Skoog (1957) found that IAA is involved in the first two steps of cell division (i.e., in DNA synthesis and mitosis) and that the last step (i.e., cytokinesis) is controlled by kinetin. It has been suggested that the adenine moiety of the kinetin molecule is essential for cell division.

2. CELL ELONGATION. Besides auxins and gibberellins, kinetin also promotes cell elongation. Such promotion after kinetin treatment has been observed in tobacco pith cultures (Glasziou, 1957), tobacco roots (Arora et al, 1959) and bean leaf tissues (Powell and Griffith, 1960). Since cell elongation induced by kinetin has been well established, the kinetin should not be regarded as exclusively a cell division factor.

3. ROOT GROWTH. Kinetin is capable of stimulating as well as inhibiting root development. Skoog and Miller (1957) found stimulatory effect of kinetin, when applied along with IAA, on root initiation and development in stem callus cultures. Similarly, kinetins also induced increase in dry weight and elongation of the roots of lupin seedlings (Fries, 1960).

4. SHOOT GROWTH. The callus tissue of tobacco can be kept in an undifferentiated state so long as the proper balance of IAA and kinetin is maintained. If, however, the amount of kinetin is increased, leafy shoots are initiated to develop. Bean seedlings, soaked in kinetin solution, also showed an increase in dry weight and a marked elongation of stem and petioles (Miller, 1956).

Contd...P/15.

5. MORPHOGENESIS. Cytokinins can cause the formation of organs in a variety of tissue cultures. For instance, Skoog and Miller (1957) observed that tobacco pith callus can be made to develop either buds or roots by changing the relative concentrations of kinetins and auxins. High kinetin and low auxin contents result in the production of buds. In reverse condition (high auxin and low kinetin), however, the roots appear on the pith.

The kinins also stimulate the production of buds in leaf segments of various plants such as Saintpaulia innantha, Bryophyllum sp and Begonia sp.

In addition to the root and shoot differentiation, the cytokinins also bring about other morphogenetic responses. These are :

(a) maturation of proplastids into plastids
(b) differentiation of tracheids
(c) induction of parthenocarpy
(d) induction of flowering

6. COUNTERACTION OF APICAL DOMINANCE. As discussed earlier (refer page 490), the auxins emanating from the apical bud inhibit the growth of lateral buds (apical dominance). Wickson and Thimann (1958) studied the antagonistic effect of auxin and kinetin in apical dominance using pea stem sections in culture solutions. They found, as might be normally expected, that the growth of lateral buds is inhibited when the culture medium contain IAA and is uninhibited when the culture medium does not contain IAA. They further noted that addition of kinetin, along with IAA, stimulates the growth of lateral buds.

Contd...P/16.

The above workers also conducted experiments with entire shoots, i.e., with the apcial bud intact. So long as the apical bud is present, the lateral buds do not develop but removal of the apical bud leads to the stimulation of growth of the lateral buds. If, however, the intact shoot is soaked in kinetin solution, the inhibition of lateral buds is checked to a great extent or in other words the lateral buds tend to develop, Although less vigorously, as if the apex of the shoot has been cut off. The above findings point out towards the possibility of controlling apical dominance by maintaining a proper balance of concentrations between IAA and the endogenous kinetin-like substances.

7. BREAKING DORMANCY OF SEEDS. Cytokinins are also effective in breaking seed dormancy in lettuce, tobacco, white clover and carpet grass. Thimann (1963) suggests that the site of cytokinin action in such cases is the cotyledon. Furthermore, the inhibitory effect of infra-red light on germination of lettuce seeds is also alleviated by kinetin treatment.

The seeds of parasites such a Striga asiatica require the presence of host plant for germination. But when treated with kinetin, the seeds germinate even in the absence of their host.

8. DELAY OF SENESCENCE (= Richmond-Lang effect).

The term senescence refers to the ageing of the leaves which is associated with the loss of chlorophyll and the breakdown of proteins. Richmond and Lang (1957) showed that the senescence in the detached leaves of Xanthium could be postponed for many days by kinetin treatment. This effect of kinetin in retarding senescence (or ageing) is known as

Contd...P/17.

Richmond-Lang effect. According to Mothes and Engelbracht (1961), the cytokinins have the ability to attract certain substances including auxins and to prevent the movement of leaf components out of the treated area. However, the mobilizing effect of cytokinin may actually induce senescence in other parts of the plant. Osborne (1962) suggests that the high protein content in kinetin treated areas is probably due to enhanced protein synthesis than their breakdown. The protein synthesis, in its turn, is dependent on RNA synthesis, a process governed by kinetins.

A correlation between the age of the leaf and the kinetins has been established. Mature leaves of tobacco respond more vigorously to kinetin treatment in delaying senescence than the young leaves.

9. ROLE IN ABSCISSION. Cytokinins can accelerate as well as retard the process of abscission in leaf petioles depending on the site of their application (Osborne and Moss, 1963). In explant petioles of Coleus blumei, Gupta and Kaushik (1969) reported accelerated abscission on kinetin application.

OTHER NATURAL GROWTH HORMONES IN PLANTS

1. ETHYLENE

Although the presence of ethylene has been shown in certain fungi (Penicillium digitatum, Alternaria citri) and in the leaves, flowers and fruits of many higher plants since long, its recognition as a natural plant growth hormone has only recently been confirmed by Pratt and Goeschl in 1969. Ethylene also occurs in minute quantities in city gas and in tail gases of blast furnaces. It is a gas of peculiar odour and is sparingly soluble in water but a little more in ethanol and ether. It is inflammable and hence the ignition

Contd...P/18.

of a mixture of ethylene with air leads to explosion.

2. ABSCISIC ACID

The structure of Abscisic acid is given in Fig. Eagles and Wareing (1963) isolated an inhibitor from the birch leaves held under short day conditions. When this substance was reapplied to the leaves of birch seedlings, apical growth was completely arrested. As this substance induced dormancy, they named it as dormin. Later in 1965, Ohkuma et al isolated an inhibitor from cotton fruits and named it abscisin II. The same year, Cornforth and his associates isolated a growth inhibitor from sycamore and pointed out that both dormin and abscisin II are identical. Abscisin II is peculiar in that it is effective in much lower concentration than phenolic inhibitors and is accumulated under short day conditions.

Since its isolation and characterization, abscisin II has been found to perform a number of physiological functions:

1. It accelerates abscission and senescence in many plants such as cotton.
2. It inhibits seed germination in ash and lettuce.
3. It inhibits flower induction in Lolium sp.
4. It inhibits growth of IAA-induced oat coleoptiles.
5. It inhibits GA-induced enzyme synthesis in barley aleurone layers.
6. It inhibits completely the sprouting of potato buds.

Dr.U.K.Nanda
Reader in Zöology

Introduction

Fertilisation brings about some change in the egg structure which undergoes cleavage. We come across diverse pattern of cleavage in the eggs of different animals, depending upon the amount of yolk it contains. Even in the three subdivisions of the class Mammalia we find there exist some difference in their mode of development.

a) Subclass Prototheria: Eggs have large amount of yolk, showing essentially reptilian pattern of development.
   Eg: Ornithorhynchus Echidna
b) Subclass Metatheria(the marsupials): The developing embryos receive nourishment from the mother in the uterus though this adaptation is not so well developed as in Eutheria. The yolk though present is ejected at the beginning of cleavage.

With the disappearance of yolk, mammalian eggs have reverted to complete cleavage, but subsequent development bears ample evidence of the former presence of yolk and in many respects the morphogenetic process resemble those in meroblastic eggs with a discoidal type of cleavage.

Cleavage

1. Soon after fertilisation cleavage starts.
2. Cleavage is complete, holoblastic blastomeres are more or less of equal size but the cleavage is not as regular as in oligolecithal eggs.
3. The first cleavage is vertical and divides the blastomeres into two unequal blastomeres.
4. Synchronization of the mitosis in the blastomeres is lost very early.
5. Second cleavage is also vertical but at right angles to the first cleavage. One of the blastomeres divides into two resulting in the occurrence of 3 blastomeres.
6. The third cleavage is horizontal.
7. Subsequently five, six and seven blastomeres and so forth are formed.

Speed of Cleavage

8. Overall spreed of the cleavage is much lower than in many other animals. Several hours elapse between successive divisions.

Formation of morula

9. As a result of cleavage a solid mass of cells, a morula, in which some cells (inner mass of cells) are large and lie inside completely cut off from the surface by a enveloping cells, which are smaller.

10. In due course the superficial cells join to form a distinct epithelial layer.

11. This layer gives rise to parts(the embryonic membra and serves to

a) attach the embryo to the uterine wall.

b) mediates in the supply of nourishment to the embryo from the maternal body via the placenta.

12. The outer layer of the mammalian embryo is known as the trophoblast.

13. The cells lying in the interior are known as inner cell mass and it is these cells that provide material for the formation of the embryo proper. They are therefore be referred to as the formative cells.

14. The two kinds of cells are distinguishable rather early by certain physiological properties, which may be shown by using specific staining methods.

15. 'Cells of inner cell mass have been found(in the rat) to be more basophilic than trophoblastic cells, suggesting a higher content of cytoplasmic nucleic acids(Jones-Seaton,1950) and also been found to cont the enzyme alkaline phosphatase, which is lacking in trophoblastic cells (Dalaq,1954).

Formation of blastocoel

16. Soon a cavity appears inside the compact mass of cel of morula. The cavity is formed of crevices which ap between the inner cell mass and the cells of the trophoblast.

17. Fluid is imbibed into this cavity, so it enlarges, t whole embryo becomes bloated to the same degree.

18. The trophoblast becomes lifted off the inner cell ma on most of its inner surface, and it remains attache one side only,which corresponds later to the dorsal of the embryo.

19. The mammalian embryo at this stage is called blastoc

20. The cavity of blastocyst may be compared to the blastocoel, but the embryo as a whole differs essent from a blastula, since its cells are already differe ted into two types:

a) the inner cell mass
b) and the cells of the trophoblast

21. Role of trophoblast and inner mass of cells

There is experimental evidence that the properties of inner cell mass and those of the tropholast are alrea distinctly different at this stage.

Three and a half day-old mouse embryos were cut in pieces, so that some pieces contained only inner cell mass and other consisted of trophoblast cells (Garne 1972). It was found that clumps of inner mass of cel fused together in larger masses, while clumps of trophoblast cells formed vesicles which did not adhe to one another.

22. When introduced into uteri of pseudopregnant mice, t inner cells mass did not react with the uterine wall thus they did not become implanted and thus did not develop further.

23. On the otherhand, the vesicles formed from the trophoblast, when introduced into the uteri, established connection with the uterine wall as in the development of normal embryos but later failed to proliferate. This experiment clearly reveals the roles of the two components of the early mammalian embryos.

Formation of the hypoblast

24. A layer of very flat cells appear on the interior surface of the inner cell mass, that is, on the surface facing the cavity. This layer of flat cells corresponds to the lower layer of cells of the chick blastoderm, the hypoblast. The cells respresent the presumptive endoderm or at least a part of it.

25. The origin of the hypoblast endodermal cells is a subject of some controversy. The generally accepted view is that the endodermal cells are split off from the inner cell mass.

26. There are indications, however, that the endodermal cells in some mammals such as the elephant shrew(Van der Hor.t, 1942), or even in all mammals (Dalcq, 1954) are derived from the cells of the trophoblast. Some of these, near the edge of the inner cell mass, migrate inward along the internal surface of the inner cell mass and arrange themselves in a continuous layer, spreads out later to enclose the cavity or the yolk sac.

27. Unfortunately, the method of local vital staining or marking cells could not as yet applied to study of the migration of cells, which give rise to hypoblast cells in mammals.

28. Thus, the conclusion must be reached on the grounds of difference in the staining reactions of the various cells.

a) do not have basophilic stain
b) negative reaction for alkaline phosphatase.

This suggest that hypoblast is derived from trophoblast.

29. The trophoblast corresponds in position to the chorion of the taller embryos of reptilese birds but there is a difference that chrion develops in conjunction with amnion.

30. The inner cell mass then spreads out and becomes arranged into a plate, resembling the epiblast of the blastodisc of reptiles and birds.

31. The arrangement of the formative cells in the mamamlian blastocyst is at this stage similar to that in the avian blastodisc prior to the apperance of primitive streak but under the blastodisc of mammal there is a fluid filled cavity.

32. In some mammals where the development is primitive (rabbit) the layer of trophoblast, Raubers layer, over the epiblast becomes temporarily superficial.

33. In higher mammals this layer never disappears and the formative cells are never exposed to the exterior.

34. The blastodisc consisting of epiblast and a hypoblast, becomes quite sharply delimited from the remainder of the embryo.

35. Epiblast: Consists of a thick plate of columnar cells clearly distinguishable from the flatter and more irregularly arranged cells of the trophoblast.

Hypoblast: Cells on the underside of the blastodisc may become cuboidal or even columnar and differ from extra embryonic endoderm lining the internal surface of the trophoblast. At the edge of the blastodisc. This thickening is the prechordal plate which forms the roof of the archenteron and it denotes the anterior end of the embryo.

Gastrulation

36. A primitive streak is formed and a Hensen's node is seen at the anterior end of the primitive streak.

37. Primtive streak is shorter than birds and do not surpa half the length of the blastodisc, being confined to posterior part.

Formation of Endoderm

38. The cells of the primitive streak migrate downward and sideways between the epiblast and the hypoblast and th contribute to the formation of the endoderm (" and Streeter, 1941).

Formation of mesoderm

39. The loose cells migrating sideways give rise to the la of mesoderm.

Formation of notochordal rudiment

40. The cells migrating forward from Hensen's node remain packed more closely and give rise to the "head process the notochordal rudiment.

Archenteric canal

The notochordal rudiment in some mmals (including man) is perforated by a canal starting from Hensen's node known as archenteric canal.

In other mmals, either there is no archenteric canal the canal, though present in the notochordal rudiment, does not open to the surface at Hensen's node.

Where an archenteric canal is present, its ventral wa fuses later with the hypoblast and is then perforated the archenteric cavity opens into the yolk sac cavity.

Subsequently, the notochord separates itself from the endoderm, and the endoderm closes underneath the noto chord, forming again a continuous layer.

....

THE MODERN SYNTHETIC THEORY OF EVOLUTION

Prof. N.Khattar,
Head of Science,
RCE, Bhubaneswar.

The modern, synthetic theory of evolution recognizes four basic types of processes: GENE MUTATION, CHANGES IN CHROMOSOME STRUCTURE AND NUMBER, GENETIC RECOMBINATION, and NATURAL SELECTION. Thefirst three provide the genetic variability without which change cannot take place; natural selection guides populations of organisms into adaptive channels (Figure 1.1.). In addition, three accessory processes affect the working of these four basic processes. MIGRATION of individuals from one population to another, as well as HYBRIDIZATION between races or closely related species both increase the amount of genetic variability available to a population. The effects of CHANCE, acting on small populations, may alter the way in which natural selection guides the course of evolution. The purpose of the present book is to review our knowledge of each of these processes, and to show how they are interrelated with each other. The more we know about the four basic processes, the less reason we have for believing that any other basic processes remain to be discovered. We do not need to search any more for hidden causes of evolution. Nevertheless, we do need to understand much more about the way in which known processes interact with each other.

At the outset we must recognize that at least in higher organisms, and perhaps in microorganisms as well, the three processes, mutation, genetic recombination, and natural selection, are equally

indispensable for evolutionary change to take place. Speculations as to which of the three is the most important are completely pointless. The best way to understand their interrelationships is to recognize that all populations of sexually reproducing organisms contain a large "gene pool" of genetic variability. Like a natural pool of water, the gene pool maintains a dynamic equilibrium between inflow and outflow of genes, and may become larger or smaller, depending upon various external and internal factors. Genes may be added to the pool (1)by immigration from other gene pools, which requires crossing or hybridization between immigrants and old residents of the population; or (2) by mutation, followed by spread of the mutant allele through the population . Genes are removed from the pool chiefly by (1) natural selection, which constantly cleanses the pool of unfavourable mutations and builds up adaptive complexes of genes, and (2) chance elimination of alleles, which takes place in small populations or during reductions of population size. Genetic recombination, following the principles of Mendelian heredity, is constantly reshuffling the genes in the pool, presenting new combinations for acceptance or rejection by natural selection. Its importance lies in the fact that adaptiveness of an individual rarely if ever depends upon the independent action of individual genes or gene mutations. Due to constant interaction between genes at different loci, or EPISTASIS, the adaptive value of most genes that are retained in populations depends upon their ability to form favourable combinations with other genes.

Natural selection, which results from interactions between populations and their environment, may either stabilize gene composition by eliminating most or all immigrants and mutants, or change it in various ways. Evolution takes place through alterations of the frequency of genes and gene combinations in the population, brought about by natural selection. Finally, reproductive isolation, which includes all the barriers to gene exchange between populations, has a canalizing effect. Since the richness and organizational complexity of the gene pool make possible several different responses to the same kind of environmental change, populations that are reproductively isolated from each other are almost certain to evolve in different directions, while those that are not so isolated because of gene exchange, will evolve in the same direction.

.....

ORIGIN OF SPECIES

Prof.N.Khattar,
Head of Science Deptt.

The medium of evolution is the population, a geographically localized aggregation of members of a given species. The raw materials of the evolutionary process are the inheritable variations which appear among the individuals of such a population. And the mechanism of evolution may be described as natural selection acting on the inheritable variations of a population.

In a population, the members interbreed preferentially with one another and they also interbreed occasionally with members of neighbouring sister populations (see Chap.8). The result of the close sexual communication within a population is a free flow of genes. Hereditary material present in a part of a population may in time spread to the whole population, through the gene-pooling and gene-combining effect of sex. Therefore, in the course of successive sexual generations, the total genetic content of a population may become shuffled and reshuffled thoroughly. We may say that a population possesses a given gene pool and that the interbreeding members of the population have free access to all components of that pool. Moreover, inasmuch as sister populations are in occasional reproductive contact, the gene pool of one population is connected also to the gene pools of sister populations. In this way, the total genetic content of an entire species continues to be

31.1 Concept of a gene pool. In a species, genes flow within and between populations. The total gene content of the species thus represents a gene pool to which

all members of the species have access. Genes normally cannot flow between the gene pools of two different species, shuffled about among the member organisms (Fig.31.1).

Evolution operates via the gene pools of populations. We already know from Chap.30 how changes in genetic systems, hence inheritable variations, may arise: by sexual recombination and by mutation. In each generation, some individuals may appear exhibiting new trait variations, as a result of either recombinational or mutational process (see, for example, Fig.8.1). If these variant organisms survive and have offspring of their own, then their particular (mutational) genetic innovations will persist inthe gene pool of the population. In the course of successive generations, the genetic novelty may spread to many or all members of the population.

Whether or not such spreading actually takes place depends on natural selection. This term is synonymous with differential reproduction. Either "natural selection" or "differential reproduction" means simply that some individuals of a population have more offspring than others. Clearly, those leaving more offspring will contribute a proportionately greater percentage of genes to the gene pool of the next generation than those leaving fewer offspring. If, therefore, differential reproduction continues in the same manner over many generations, the abundant reproducers will contribute a progressively larger number of individuals to the whole population.

As a result, their genes will become preponderant in the gene pool of the population (Fig. 31.2).

Which individuals leave more offspring than others? Usually, but by no mans necessarily, those that are best adapted to the environment. Being well adapted, such individuals on the whole are healtheir and better fed, may find mates more readily, and may care for their offspring appropriately. However, circumstances may on occasion be such that comparatively poorly adapted individuals have the most offspring. Instances of this are sometimes encountered in human populations, for example. In any event, what counts most in evolution is not how well or how poorly an organism copes with its environment, but how many offspring it manages to leave. The more there are, the greater a role will the parental genes play in the total genetic content of the population. By and large, the well-adapted organism contributes most to the gene pool.

Therefore, if an inheritable variation appears in an organism and if, through differential reproduction in successive generations, the progeny of that organism becomes numerically more and more abundant, then a given genetic novelty will spread rapidly throughout the population. As a result, a trait variation originating in one organism will have become a standard feature of the population as a whole.

This is the unit of evolutionary change. Many such unit changes must accumulate in a population before the organisms are sufficiently altered in structure or function to be established as a new species.

All evolution operates through the basic process just descsribed. In brief, it consists of:

1. appearance of inheritable variations by sexual recombination and mutation

2. spreading of these variations through a population by differential reproduction in successive generations.

In as much as inheritable variations originate at random, evolutionary innovations similarly appear at random. But in as much as the best reproducers are generally the best adapted, evolution as a whole is directed by adaptation and is oriented toward continued or improved adaptation. It is therefore not a random process.

Note that, in this modern view of evolution, natural selection is fundamentally a creative force; for its important effect is to spread genetic novelty, hence new traits, through a population. It is also a peaceful force, involving reproduction, not "struggle for existence" or "survival of the fittest." Organisms actually struggle rather rarely. Indeed, animals try to avoid struggle and attempt to pursue life as inconspicuously as possible, eating when they can, reproducing when they can. And plants have never been seen to engage in struggles at all. Moreover, natural selection does not "eliminate the unfit". The "fit" may be the mightiest and grandest organism in the population, but it might happen to be sterile. And the "unfit" could be a sickly weakling, yet have numerous offspring. The point is that neither

"survival" nor "elimination" is actually at issue. The only issue of consequence here is comparative reproductive success. Indirectly, to be sure, health, fitness, and even actual physical struggles may affect the reproductive success of organisms. To that extent such factors can have evolutionary consequences. But what in Darwin's day was regarded as the whole of natural selection is now clearly recognized to have only a limited, indirect effect on evolution. The whole of natural selection, directly and indirectly, undoubtedly is differential reproduction.

THE GENETIC BASIS

The Hardy-Weinberg Law

From the preeeding, we may conclude that evolution is characterized by a progressive change of gene frequencies. Thus, inthe course of successive generations, the proportion of some genes in the population increases and the proportion of others decreases. For example, a mutation mau at first be represented by a single gene, but if by natural selection this mutation spreads to more and more individuals, then its frequency increases whereas the frequency of the original unmutated gene decreases. Clearly, the rates with which gene frequencies change will be a measure of the speed of evolution. What determines such rates?

Suppose we consider a large population in which two alleles, A and a, occur in certain frequencies. In such a population, three kinds of individuals will be found, namely, AA, Aa, and aa. Let us assume that the numerical proportions happen to be

AA	Aa	aa
36%	48%	16%

Assuming further that the choice of sexual mates is entirely random, that all individuals produce roughly equal numbers of gametes, and that the genes A and a do not mutate, w e may then ask how the frequency of the genes A and a will change from one generation to the next.

Since AA individuals make up 36 percent of the total population, they will contribute approximately 36 percent of all the gametes formed in the population. These gametes will all contain one A gene. Similarly, aa individuals will produce 16 percent of all gametes in the population, and each will contain one a gene. The gametes of Aa individuals will be of two types, A and a, in equal numbers. Since their total amounts to 48 pe cent, 24 percent will be A and 24 percent will be a. The overall gamete output of the population will therefore be

parents	gametes	parents	gametes
36% AA	36% A	16% aa	16% a
48% Aa	24% A	48% Aa	24% a
	60% A		40% a

Fertilization now occurs in four possible ways: two A gametes join; two a gametes join; an A sperm joins an a egg; and an a sperm joins an A egg. Each of these possibilities will occur with a frequency dictated by the relative abundance of the A and a gametes. There are 60 percent A gametes. Accordingly, A will join A in 60 percent of 60 percent of the cases, that is,

60 X 60, or 36 percent of the time. Similarly, A sperms will join a eggs in 60 X 40, or 24 percent of the cases. The total result:

Sperms	Eggs		Offspring
A	A	60 X 60	36% AA
A	a	60 X 40	24% Aa
a	A	40 X 60	24% Aa
a	a	40 X 40	16% aa

We note that the new generation in our example population will consist of 36 percent AA, 48 percent Aa, and 16 percent aa individuals. These are precisely the same proportions we started with originally. Evidently, gene frequencies have not changed.

It can be shown that such a result is obtained regardless of the numbers and the types of gene pairs considered simultaneously. The important conclusion is that, if mating is random, if mutations do not occur, and if the population is large, then gene frequencies in a population remain constant from generation to generation. This generalization is known as the Hardy-Weinberg law. It has somewhat the same central significance to the theory of evolution as Mendel's laws have to the theory of heredity (Fig.31.3).

The Hardy-Weinberg law indicates that, when a population is in genetic equilibrium, that is, when gene frequencies do not change, the rate of evolution is zero. Genes then continue to be reshuffled by sexual recombination and, as a result, individual variations continue to originate from this source. But the overall gene frequencies do not change.

Of themselves, therefore, the variations are not being propagated differentially. Evolution consequently does not occur.

What does make evolution occur are deviations from the "ifs" specified in the Hardy-Weinberg law. Thus, mating is decidedly not random whenever natural selection takes place; genes actually do mutate; and populations are not always large. Singly and in combination, these three factors may disturb the genetic equilibrium of a population and may produce evolutionary change.

SPECIATION

The key process to be explained is how unit evolutionary changes in a population eventually culminate in the establishment of new species and higher taxonomic categories. As already shwon in Chap.8, a species may be defined as a collection of populations within which reproductive communication is maintained by inter-breeding. We may now define a species alternatively as a group of populations sharing the same gene pool (see Fig.31.1). Within the pool a free flow of genes is maintained, but genetic flow between two such pools does not occur; a reproductive barrier isolates one species from another. The problem of speciation, therefore, is to show how reproductive barriers arise.

Geographic barriers between sister populations usually develop before biological reproductive barriers come into existence. Among geographic barriers, distance is probably the most effective. Suppose that, in the course of many generations, the populations of a given species grow in size and number and that, as a result of the increasing population pressure, the organisms radiate into a progressively larger territory. In time, two populations A and Z at opposite ends of the territory may be too far apart to permit direct interbreeding of their members. Although gene flow still takes place via the interconnecting populations between A and Z, individuals of A and Z no longer come into reproductive contact directly (Fig.31.7).

It is then almost certain that, by chance, different genetic innovations arise in A and Z and that different ones will be propagated within A and Z by natural selection. Such an effect will be particularly pronounced if the environments of A and Z are or become more or less different. If now the evolutionary changes within A and within Z occur faster than the speed of genetic flow between A and Z, then A and Z will actually become progressively different in structure or function. These two populations thus may come to represent two distinct subspecies (Fig. 31.8).

Geographic isolation here has set thestage for the development of initial differences between members of A and Z. If the differences accumulate, they may eventually become so great that gene flow between A and Z will stop altogether. For example, population A (or Z) may undergo a change in the reproductive organs such that mating with neighbouring populations becomes mechanically impossible. Or the protein specificities of A may so change that the gametes become incompatible with those of neighboring populations. Or the time of the annual breeding season in A may become advanced or delayed relative to that of neighboring populations. Or the individuals of A may become changed psychologically, so that they no longer accept mates from neighboring populations. Biological barriers of this sort will interrupt all gene flow between A and Z. These subspecies, isolated reproductively, then in effect will have become two different species (Fig.31.9).

Although an initial isolation due to distance is probably the most common kind, other forms of geographic isolation are encountered as well. The development of terrestrial islands surrounded by water or of aquatic islands surrounded by land, the interposition of a forest belt across a prairie or of a prairie belt across a forest, the appearance of mountain barriers, river barriers, temperature barriers, or of many another physical barrier, each may result in geographic isolation. With reproductive contact then lost between two populations, evolution in each may henceforth follow entirely different courses. In effect, the parental species will become split into two new ones. At first, the descendant species will still be rather similar structurally and functionally. In time, however, evolutionary changes are likely to introduce progressively pronounced differences, including biological barriers to interbreeding. These add to and reinforce the environmental ones already in existence.

In two just-formed sister species, interbreeding often may still take place if the isolating condition is removed, but in nature such removals do not normally occur. Therefore, when two different species do not interbreed in nature, this does not always mean that they cannot interbreed. In many cases, members of different species may be brought together in the laboratory and there they interbreed perfectly well. For example, swordtails and platys, two species of tropical fish (Fig.31.10), may under certain conditions have offspring inthe laboratory. But in nature they

almost never do because they are isolated reproductively; although they live together in the same rivers, biological barriers discourage crossbreeding. And after two sister species have been separated for long periods, interbreeding will no longer be possible even if members of the two are brought together artificially. Biological differences sooner or later become sufficiently pronounced to preclude interbreeding.

Speciation by this means is the principal way in which new species evolve. Such a process takes, on an average, about I million years. Consciously or unconsciously making use of this principle of reproductive isolation, man has been and is now contributing to the evolution of many other organisms. Here may be found direct proof that evolution actually occurs and, indeed, that it operates according to the mechanism described above.

The most ancient evolution-directigg effort of man is his successful domestication of various plants and animals. Darwin was the first to recognize the theoretical significance of domestication, and it was this , actually, which led him to his concept of natural selection. He reasoned that if man, by artificial selection and isolation, can transform wild varieties of given plants and animals into domesticated varieties, then perhaps natural selection and isolation, acting for far longer periods, can bring about even greater evolutionary transformations in nature. We know now that the domesticating process in fact does involve all the elements of natural evolution: first, deliberate

physical, hence reproductive and genetic, isolation of a wild population by man; and second, long-continued, carefully controlled, differential reproduction of individuals "adapted" to human desires, that is, of individuals exhibiting traits considered desirable by man. The result is the creation of new strains, races, subspecies, and even species (Fig.31.11).

Furthermore, during the last few decades, rather rapid, man-directed evolution has taken place among certain viruses, bacteria, insects, various parasites, and other pest organisms. These live now in an environment in which antibiotics and numerous pest-killing drugs have become distinct hazards. And the organisms have evolved and are still evolving increasing resistance to such drugs. Indeed, the very rapid evolution of viruses and bacteria becomes a problem in research; laboratory populations of micro-organisms may evolve resistance to a drug even while the drug is being tested. Because micro-organisms have exceedingly short generation times, because their populations are physically small, compact, and easily reared, and because high mutation rates may be induced readily by X rays, they have become favorite test objects in evolution experiments.

.....

PLANT SPECIES

Dr. G. V. Gopal

ORIGIN OF NEW SPECIES

A species is not a static unit. Through time, it gets modified into new forms (Fig. 4.1). New species arise either by the slow transformation of existing species or existing species populations, or abruptly as a result of hybridization and polyploidy or through special evolutionary mechanism called catastrophic selection or even through counterfeit hybridization.

1. GRADUAL SPECIATION :

(a) Phyletic speciation. A species with restricted distribution may get gradually transformed into new species by the development of new genetic material produced by mutations and by continual incorporation of favourable genetic changes in its gene pool. The net result is that in place of one old species there is one new species. This process of gradual transformation of an old species into a new one is known as phyletic speciation.

(b) True speciation. In true speciation physical or biological isolation develops in a widespread species and in due course of time due to development of new acceptable genetic material and its continual incorporation there takes place slow divergence of isolated populations into two or more species (Fig. 4.2).

It was Mortiz Wagner who put forth a hypothesis of true speciation in 1868 which is known as geographical theory of speciation or even Allopatric theory of speciation. According to this hypothesis the first step in true speciation is the separation of original gene pool into two or more parts (Shrinkage) as a result of reproductive isolation brought about by changes in the environment or may be the consequence of long-range dispersal in which case, as has been emphasized by Stebbins (1960), self-pollinating species have a greater chance of successful establishment. The resulting gene pools in a bid

Contd...P/2.

to adjust themselves to their surroundings evolve in different directions. Gradually they become morphologically and physiologically quite different. This process is known as differentiation. As a result of reproductive isolation and differentiation the fragmented parts of the original gene pool come to occupy a definite geographic range. Sometimes as a consequence of further environmental changes an expansion of this range may take place resulting in overlapping. The overlapping may also result from migration of different populations into a previously unoccupied area (Fig. 4.2). It is quite possible that the two populations that happen to come together due to overlapping might have already differentiated physiologically and genetically to a degree that they are unable to form viable zygotes. In such an instance speciation process has already been completed without interaction. Conversely, the two populations may have almost similar ecological requirements and potentiality for hybridization. In that case, they will compete with each other. One population may outcompete the other restoring the original non-overlap range. Or, the two populations, as a result of further selection, may divide the environment in a way that competition is wanting and simultaneously some sort of breeding barrier is established restricting the formation of hybrids. Or, in an overlapping range/area two populations may hybridize to form one interfertile population. In fact, whenever there is competition between two populations they always tend to displace each other, that is, requirements of one or both populations change in a way that reduce competition. It has aptly been put forth by F.G. Gause (1934) in his 'Exclusion principle' which states, "No two forms can share exactly the same environmental requirements for an indefinite period of time, eventually one form will replace the other."

Thus, the two most important steps in the process of true speciation are reproductive isolation and differentiation. Sometimes secondary merger does take place but it is not an essential step of the process.

2. **ABRUPT SPECIATION**

Abrupt speciation usually occurs either as a result of polyploidy, catastrophic selection or counterfeit hybridization.

Contd...P/3.

POLYPLOIDY

This process results in the multiplication of genome or chromosome number of a plant. It has been observed that mostly resultant polyploid individuals are incapable of forming fertile offspring with individuals of the parental stock. The resultant polyploid individuals thus acquire instant reproductive isolation and if these are able to exploit an ecological situation to their benefit, the result is the formation of a new species. W.H. Lewis, (1967), however, asserted that a kind of ecological requirement need not follow chromosome doubling. This generalization was based on his observations that 2x and 4x cytotypes of Claytonia virginica, Hedyotis purpurea, H. longifolia and Oldenlandia capensis could grow only a few meters apart on ecologically similar niches. This process indeed has played a major role in the evolution of angiosperms where about 40% of the total species are polyploid. Four main types of polyploids recognized are autopolyploids, true or genomic allopolyploids, segmental allopolyploids and autoallopolyploids (Fig. 4.3) (cf. Stebbins, 1960). A large number of taxa once regarded to be autopolyploids are suspected to be of hybrid origin. Autopolyploids, in general, look remarkably similar to their diploid progenitors. Segmental allopolyploids simulate autopolyploids in close morphological resemblance to diploid forms and in displaying of multivalent configuration at meiosis, and are mostly confused with them. Nothing can be said with certainty regarding their actual ancestry until both of its parents have been identified through hybridization experiments. Segmental allopolyploids possess the ability for genetic segregation with regard to morphological differences and also chromosomal differences forming sterility barrier between the parental species. Further, segmental alloploidy is an unstable condition which, guided by selection for fertility, will either evolve in the direction of auto or typical allopolyploidy. If chromosomes of the parental species were largely similar and differed by one or two small non-homologous segments, selection would tend to eliminate these non-homologous segments and favour evolution in the direction of autopolyploidy. If the situation

Contd...P/4.

was other way round, that is, chromosomes differing initially by numerous and large segments then selection would favour further differentiation of such chromosomes through mutations and chromosomal rearrangements and elimination of multivalent formation, thus, directing them towards typical allopolyploidy. Segmental allopolyploids sometimes form partly fertile hybrids through backcrossing with autopolyploid derivatives from either of their parental species. Typical allopolyploids usually stand out as clearly marked species as hybrids resulting from their backcrossing to either diploid parent or to their autopolyploid derivatives are usually partly and sometimes completely sterile. Apart from being fully fertile allopolyploids are strongly isolated from, and also morphologically discontinuous with their closest relatives. Thus, polyploid species complexes which pose difficult problems to systematists contain either segmental allopolyploids or autopolyploids or both. Autoallopolyploids exist only at the level of hexaploidy or higher. A very strong resemblance may result between an autoallopolyploid and one of its diploid ancestors if it contains two or more genomes derived from one species and only one genome from the other.

CATASTROPHIC SELECTION :

In 1962 Harlan Lewis proposed a special evolutionary mechanism which does not involve the process of polyploidy and leads to rapid speciation resulting from genetic isolation with little or no morphological differentiation. He observed that three species of Clarkia (Onagraceae) e.g C. franciscana, C. rubicunda and C. amoena were morphologically quite similar (Lewis and Raven, 1985 a, b). They crossed these species in all combinations to observe the fertility of hybrids and to note the differences in chromosome architecture as exhibited by chromosome pairing at meiosis. In every case nearly 2% of the pollen were found to be fertile and chromosome pairing in hybrids revealed that all the species differed drastically from each other in arrangement of the homologous segments. C. franciscana differed from C. rubicunda by at least three large translocations and four paracentric inversions and from

Contd...P/5.

C. amoena by at least two translocations and two or more paracentric inversions. The species C. amoena differed from C. rubicunda by three translocations and two inversions. Thus, the three species of Clarkia, which were morphologically very similar, were found to be dissimilar in respect of arrangement of genes on the chromosomes. The genetic isolation results from this dissimilarity. How such chromosomal alterations arise ? To explain this Lewis put forth the hypothesis of catastrophic selection according to which under certain unknown conditions (environment stress such as drought etc.) drastic chromosomal rearrangements may take place in a plant and if such a plant becomes detached from its parent population and is capable of establishing a population of its own with new chromosomal rearrangements then it results in the formation of a population which is genetically isolated from the ancestral population from its very inception, leading to the formation of a new species. What are the actual forces responsible for such drastic changes in some and so little in other taxa is still a mystery ! Probably a major force resides in genetic system of each species which controls and determines the way a species reacts to the environmental changes.

COUNTERFEIT HYBRIDIZATION :

It also serves as an ideal isolating mechanism (de Wet et al., 1984). The process involves non-random transfer of genes or non-random incorporation of DNA fragments from a non-functional sperm of a taxon into specific position of the genome of parthenogenetically developing egg of another taxon. How all this happens is still not known. While conducting hybridization experiments, with Tripsacum serving as female parent and Zea as male parent, de Wet and coworkers noticed that in Tripsacum some of the maternal offsprings resulting from parthenogenetic development of non-reduced female gametophytes though contained 2n = 36 Tripsacum chromosomes, yet were hybrids rather than maternal in phenotype. Such hybrids were termed counterfeit hybrids. In tripsacum genome Zea material got incorporated without true fertilization between Tripsacum egg and Zea sperm. These hyprids differed

Contd...P/6.

from Tripsacum parent both in phenotype and cytological behaviour. They produce sterile offspring when crossed with their maternal Tripsacum parent. Tripsacum cundinamarce (2n = 36) is believed to be a counterfeit hybrid between T. dactyloides var. meridonal and Zea mays that served as the pollen parent.

SPECIATION IN VEGETATIVELY REPRODUCING PLANTS

In vegetatively reproducing plants which include most of the monocotyledonous taxa sexual reproduction is of secondary importance and very often even absent in them. In most of the members of Liliaceae and Amaryllidaceae flowers are formed but meiosis is irregular resulting in sterile pollen and no seed set. Does this mean that such taxa have reached a static step in evolution and have lost the capacity for speciation with loss of sexual reproduction ? In nature and even under cultivation we observe that majority of such taxa are capable of producing numerous new variants indicating that they must possess some effective means of speciation unrealated to sexual reproduction. In the somatic tissue of some of the varieties of Caladium bicolor (2n = 28), a member of the family Araceae, Sharma and Das (1954) observed some cells with either varying chromosome number and/or chromosome structure from the normal ones. Such variations were always found to be at random i.e. without any zonation. Variations in chromosome structure could arise as a result of chromosomal rearrangements including deletions, duplications, inversions and segmental interchanges; and variations in chromosome number through non-disjunction, somatic reduction and possibly through partial endomitosis. Further abnormal karyotype in one of the varieties was found to be normal karyotype of another variety. It was, therefore, suggested that if an abnormal nucleus with numerically or structurally altered karyotype happens to enter the growing apex which gives rise to a daughter shoot through vegetative means the new shoot would comprise cells with altered karyotype. Such a shoot on detachment from the parent plant would give rise to a completely new form. As sexual reproduction is absent, such abnormalities are not lost through gametic inviability

Contd...P/7.

(Sharma, 1956). But numerical alterations are sure to upset the nucleocytoplasm ratio which in turn is likely to affect the metabolism of the cell. It has been suggested that such an imbalance at individual cell level is rather advantageous to such plants and facilitates further irregularities leading to somatic mutation.

ECOLOGICAL PROPERTIES OF A SPECIES

If we study the performance of plants of a particular species against any single environmental gradient we always get similar graphs as depicted in Fig. 4.5.

Each species has its own range of tolerance. This represents the range within which individuals of a particular species are able to survive with reference to particular environmental gradient. Another point which emerges is that all plants of a species, which are being tested against an environmental gradient, do not grow with equal vigour in all parts of their tolerance range. Within central part of their range plants normally grow most vigorously decreasing progressively towards tolerance limits where it becomes zero. The portion of the tolerance range where vigour is greatest is termed ecological optimum of that species. Comparable responses will be observed if the same species is examined along other environmental gradients provided its individuals are sensitive to such variables. The separate responses will comprise a great variety of tolerance ranges, ecological optima and vigour within these. The sum of tolerances along all gradients to which a particular species is sensitive has been termed ecological amplitude of the species. In fact, each species has its own tolerance range, ecological optimum and its own ecological amplitude. More the ecological amplitude of a species, more successful it is in nature. For its survival, however genetic variability is imperative. This results from genetic recombination or continuous interbreeding within an interbreeding population. A species population may survive some new environmental stress if sufficient genetic variability exists within it to ensure that some gene combinations impart drought resistance. Genetically identical population, on the contrary, would be faced with extinction if the single gene combination present did not impart drought resistance.

—o—

Dr.G.V.Gopal,
Lecturer in Botany

The factors for modification can be broadly classified in three major categories namely: i) Environmental , ii) Genetical iii) physiological modification or adaptation of plants due these three major components.

Environmental Factor: The Environment as such is very difficult to define shortly. Environment consists of Edaphic, climatic, hydrological, Biotic as such. According to Hutchinson,1951,1958 introduced the concept of ecological niche referring it to the totality of biotic and abiotic factors to which a given species is uniquely adapted.

Periodicity of season also plays a dominant role in the phneology of the plant, same species collected from two different localities is not alike because the ecological factor at both places is not same.

Light Factor

Light Duration: Photoperiodism is characterised by great ecological significance. It explains why many plants in tropics where light period is almost constantly 12 hours, flower throughout the year and like wise, why so few plants in temperate, latitudes leave this characteristic. penomena It is also apparent that carticspecies must be long-day plants. They rarely flower when further south. Species requiring high temperature and long days to mature are definitely limited in their northern range.

Light quality: The quality of light is modified by clouds and fog. Water vapours absorb infrared light and from water-surface blue-green rays are mostly reflected or transmitted so that bodies of water appear blue or greenish blue. Red rays induce a great development of tissues and cause the cells to elongate very much. Blue-violet rays on the other hand inhibit growth and keep the cells small. Red and blue light are the most effective in photosynthesis by green plants. UV-rays have injurious effect upon cells and plant growth and in total the plant modification leads to varieties.

Phytochemical variation due to UV radiation frequency/intensity

Ultraviolet radiations have injurious effect on plant growth and it is therefore unfortunate that most of the ultra-violet radiation is absorbed by the ozone layer in the upper part of the atmosphere. In alpine regions, the intensity of ultra-violet radiation is much higher than at sea-level and is responsibile for the development of flavours and anthocyanins in alpine plants.

Precipitation:

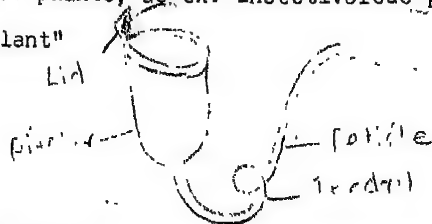
The principal forms of precipitation are rainfall, hail, snow and dew of these,rainfall is the most important as most plants absorb water from the soil. If the water level varies then the type of vegetation also varies as xerophyte ....

Mesophytes ..... and free floating hydrophyte ... submerged hydrophyte.

but some plants have modified in xxx such way their organ structure to absorb this atmospheric water directly as in the case of Lichens, orchids, epiphytic plants. Valamens roots having valamentissue is developed in this group of plants.

Wind: Wind has both a direct and an indirect effect on plant life. The direct effect of strong winds is mechanical and consists in uprooting trees and breaking off branches and twig Strong winds also cause permanent curvatures in plants on ecposed places.

Altitude: Topographic factor, the ffect of altitude is best seen in mountains. The climate changes as we ascend, becoming progressively cooler. There is greater wind action greater altitudinal varaation leads the change in vegetation of that area. If the soil is deficient of N_2 - then also it leads to the production or gives an opportunity to the development of community of plants, as ex: Insectivorous plants Nepethces "Pitcher plant"

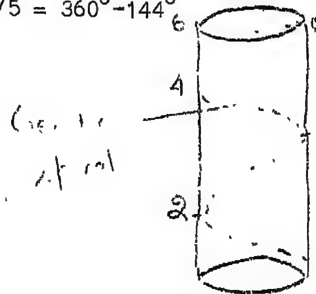


Light has as factor has many other effect on plant modification as photosynthesis is the most important biological activity. Phyllotaxis is an important phenomenon in plants which occurs in systematicx manner. Phyllotaxis means "spreading of leaves are otherwise" modern terminology says as Fibonacci series. They can

distichous due to some special types of chemical and particularly in case Psidum the plants exhibit mimicry Mi- . . ''.

i.e. 13, 57, 86, 42 i.e. 1/2 = 180^0

or 1,4,7,10,2,8,9,6,5,4,3 i.e. 2/5 = 360^0-144^0



Sometime the plant accumulate the alkaloids and some amino-acids like proline may act as defensive mechani sums is well known phenomenon.

In some case plant have modification of plant parts like spines throns, bristles, Pitcher, stingine hairs, Latex ect.

Genetical: In breeding, and out breeding the two different systems prevailing in higher plants are out crossing and inbreeding. In addition various asexual methods of reproduction have evolved in number of taxa. Outbreeding enhances recombination, heterozygosity, variability and evolutionary potential. Protandany and protosy are the two important floral-mechanisums which promote out breeding. Out breeding promoting systems is called as heteromorphic system.

Self incompatibility: In majority of the higher plants pollen is incapable of germinating on stigmas or styles of the plant that produce it. Fertilisation of the egg is thus prevented. This condition is known as "Physiological or genetical self incompatibility". This can be early manipulated by using some temperature treatments, irradiation. Bud pollination, mentor effects, paraffin oil, organic solvents, carbondioxide.

Inbreeding systems: Inbreeding system ensure regular and optimal seed production even in the absence of pollinations and are of considerable importance to colonizing taxa in new habitats.

That there are variations in the photosynthesis process among green plants is well known. There are three variants namely, the C_2type(Calvin cycle) C_4 type (C_4 dicarboxylic cycl and CAM (Crassulacean Acid Metabolism). The lower groups and a great majority of flowering plants have C_2 type of photosynthe process. C_4 photosynthesis evolved as a response to changes in carbon and oxygen concentrations in the atmosphere during the course of plant evolution (Smith B.M. 1976). Instead of the usual phosphoglyceric acid, the initial product of the well kn Calvin-Benson Cycle (C_3), the C_4 plants fix carbon initially i 4-carbon organic acid-oxaloacetic acid and then malic or aspar acid (Webster et al,1975). This type of photosynthesis is know occur in a large number of species in tropical and warm-to temperate regions. A rough estimate put the number of such spe as 945 spread out in 196 genera and 18 families(Ramadas and Raghavan,1980). Such plants have been designated as C_4 plants Hatch and Slack (1970). They have highly specialised chlorenc matous leaf bundle sheaths in which the chloroplasts have slightly modified granar and wall structure. These anatomical specialisations are together known as "Kranz syndrome" and the species having them are called "Kranz species"(Tregunna et al, Smith and Brown,1973).

Though restricted to a minority of angiospherms C_4 pathway exhibit interesting correlations and systematic implications. Smith and Brown (1973) have studied the Kranz syndrome in the Graminlae using carbon isotopes. In this fami is is confined to the three tropical subfamilies, Arundinoide Eragrostioideae and Panicoideae and holds promise in sorting the controversy over systematic positions of taxa like Aristi and Eragrostis(Rama Das and Raghavan,1980).

Within the genus Euphorbia, Crassulacean acid metbaolis has been detected in the subgenus Euphorbia and C_1-photosynt in subgenus Chamaesyce. Webster et al (1975) have concluded t the different subgenera with different photosynthesis strate have completely independent origin and hence warrant the tre of the subgenus chamaesyce as a distinct genus.

In Centrospermae C_4 photosynthesis is known in seven o 11 families. The basal group phytolaccaceae, significantly e C_2 photosynthesis(Marbry,1977). Other dicot families known t C_4 genera are Boraginaceae, Compositae and Zygophyllaceae. C photosynthetic pathway is functionally and phylogenetically distinct from the other two. The CAm plants are relatively p synthetically inefficient but are efficient in conserving wa

because their stomata open only at night (Mabry,1971). This type of photosynthesis is seen in members of families like Cactaceae, Aizoaceae and Euphorbiaceae.

Flavanoids have been one of the most exploited phyto-chemical character in relation to classification. Extensive surveys on the distribution patterns of these compounds have been carried out. Indeed, they will continue to be useful in classification at lower levels of taxonomic hierarchy, but such information on distribution alone would not be of great use in deducing phylogeny (Giannasi,1978b; Crawford,1978). Swain (1975) Harborne (1977) and many others have attempted to deduce phylogeny of these compounds. But, these speculations have mostly been based on their correlation with morphological or other nonchemical characters and hence has an element of circularity as Heywood (1973b) has pointed out.

MEDICINAL PLANTS

Dr.(Miss)G. Nair
Lecturer in Botany,
R.C.E.,Bhubaneswar.

The history of medicinal plants is intimately connected with the history of botany. Primitive man lived at the mercy of nature, in constant terror of diseases. From the earliest times, tribal priests and medicine men (witch doctors) used various plants, minerals and animal organs, usually in associaton with strange rituals and incantation, to drive out the evil spirits which they believed to be the cause of the disease. Astonishingly, these magical rites seemed to help . In some primitive tribes, a victim of disease was half-buried in soil for several days to exercise the malevolent spirits which had possessed him. Among the extremes of treatments was the chipping of holes in the skull to release the tormenting evil spirits. This theory of demoniacal possession lasted many centuries and exists even today in areas where people still live in primitive societies.

Records of early civilisation in all parts of the world reveal that a considerable number of drugs used in modern medicine were in use even in ancient times. The use of plants for curing various human ailments figured in ancient manuscripts such as The Bible, The Rig-Vedas, The Iliad and The Odyssey and the History of Herodotus. Over 6000 years ago, the ancient Chinese were using drug plants. The Egyptians, Babylonians, Sumerians, Greeks and Romans, all developed their respective characteristic Materia Medica. On the other side of the world, the

Aztecs, Mayans and Incas had all developed primitive medicine. Some of the ancient Egyptian text-books 'papyri' (such as the Edwin Smith Papyrus and the Ebers Papyrus), written as early as 1600 B.C. indicate that the Egyptians had an amazingly complex Materia Medica. Apart from the names of many medicinal plants then known, the papyri also included several hundred recipes or prescsriptions for various diseases. The Edwin Smith Papyrus (about 1750 B.C.) is now one of the prized collectins of the New York Academy of Medicine.

In India, the ayurvedic system of medicine has been in use for over three thousand years. Charaka and Susruta, two of the earliest Indian authors had sufficient knowledge of the properties of the Indian medicinal plants. Their medical works the Charaka Samhita and the Susruta Samhita are esteemed even today as treasures of literature on indigenous medicine.

The Greeks and Romans were familiar with many of the present day drugs as is evident from the work of Hippocrates (460-370 B.C.), Aristotle (384-322 B.C.). Theophrastus (370-287 B.C.), Pliny the Elder (A.D.23-79), Dioscorides (A.D. 50-100) and Galan (A.D.131-201). They wrote extensively about medicinal herbs, giving their names along with a description of each plant, illustrations, their putative healing properties and also complex descriptions for the preparation of medicines. Hippocrates, the 'Father of Medicine', was the first to attempt a scientific explanation for diseases. His influence remains today in the Hippocratic oath taken by young

doctors upon their graduation from medical school. Dioscorides' treatise on medicinal plants De Materia Medica remained the supreme authority for over sixteen centuries, during which the manuscript was laboriously copied and recopied with few additions. In like manner, the works of Aristotle, Galen and even Pliny were copied and handed down with increasing inaccuracies. During the Dark Ages (A.D. 400-1000) few new ideas were added. During the Middle Ages (A.D. 1000-1500) also, little significant botanical progress was made. Had biology progressed steadily from the time of Aristotle, there probably would have been no great plague and smallpox epidemics during the Middle Ages. One epidemic of the plague between 1347 and 1350 killed sixty million people in Europe, Asia and Africa.

About the beginning of sixteenth century, several herbals of considerable merit were published, such as those of Brunfels (1530), Bock (1539), Fuchs (1542), Cordus (1561) and L.Obel (1576). Although these works were a great improvement over earlier ones, they still propagated many myths and superstitions such as the 'Doctrine of Signatures' advocated by an eccentric genius Paracelsus (1493-1541), according to which all plants possessed certain signs given by God, which indicated their usefulness in treating diseases of similarly shaped organs in the human body. Plants, for example, with heart-shaped leaves were used for cardiac disorders; the sap of bloodroot (Sanguinaria canadensis L.) as a blood tonic; the walnut with numerous invaginations and convolutions for brain diseases and pomegranate

seeds for dental diseases. Particularly striking was themyth of the mandrake plant (Mandragora officinarum L.) of the family Solanaceae. The fleshy and often forked roots of this plant somewhat resemble the torso and the lower limbs of a human figure and were believed to be quite efficacious for treating various human ailments. Since ancient Greek times, the resulting decoction of mandrake roots either prepared in boiling water or in wine, has had a reputation of producing sleep. It was perhaps the first true anaesthesia and later in 1889 was found to contain a mixture of pain-deadening alkaloids (podophyllin, mandragorin and hyoscine), the most important of these being hyoscine or I-scopolamine.

With the development of synthetic drugs, plant products lost their significance. In the last few decades, however, there has been probably more interest in drugs obtained from vegetable sources than at any time in history because of the success with the antibiotics, and other plant drugs such as rauwolfia (for the treatment of mental diseases), podophyllum (a cathartic, as well as for curing cancerous tumors in mice), aloe (a cathartic, as well as for the treatment of atomic radiation burn); veratrum (hypertensive agent), peyote (psychoactive drug) and sundry others. Sapogenins (closely related to steroids), obtained from many members of Dioscoreaceae and Agavaceae, can be converted into cortisone, into male hormone (testosterone) and female hormones (estrogens and progesterone). Sapogenins have many potential uses in the treatment of rheumatoid arthritis and the female hormones are used in contraceptive pills.

From the crude beginning of the earlier physician-botanists, the study of drugs and drug plants has developed into modern pharmacognosy (pharmacon = drugs and gnosis = to know) which deals with the knowledge of history, botany (including properties and methods of preparation of drugs), preservation and commerce of crude drugs. Pharmacology is the study of the action of drugs on an organ or organism. Nature has provided a rich storehouse of herbal remedies to cure all mankind's ills. Throughout the world primitive peoples have utilised several thousands of different plants and plant products as cures for human ailments. Many of them have been rendered obsolete today because of the synthesis of their active principles. Some are widely cultivated, but most are gathered entirely in the wild state.

The information on drugs and drug plants whose efficacy in medicine has been established is available in various authentic books known as 'pharmacopoeia' and the drugs included therein are described as 'official'. The most important of these pharmacopoeia are the 'United States Pharmacopoeia'. British Pharmaceutical Codex'; 'Indian Pharmaceutical Codex'; and 'National Formulary'. These works are constantly being revised and kept up-to-date.

The medicinal value of drugs is due to the presence of certain substances such as alkaloids, glycosides, resins, volatile oils, gums, tannins, etc. Some of these are powerful poisons if administered indiscriminately, while others are dangerously habit-forming. Even the most dangerous drugs can be of value to human beings, if judiciously employed. The danger of

self medication is serious and extensive. People who believe themselves ill or simply off-colour physically or mentally, often use wonder-working drugs (happy pills) to relieve themselves from the tensions of modern living. These tranquillisers have proved so effective that their use has increased amazingly and has now outstripped all other drugs with the exception of the antibiotics. However, when used without proper medical advice, they may bring misery to millions.

The active principles of plant drugs are commonly more concentrated in storage organs. Roots, seeds, bark and leaves are much represented in the Materia Medica, flowers are less commonly used. While woods and woody parts of herbaceous stems are usually relatively inert.

Botanical drugs have been variously classified dependigg upon; the plant and plant parts from which they are derived, the disease for which they are used and their chemical nature.

We shall discuss below only some of the most outstanding drugs; other commercial drugs and drug plants will only be listed.

Atropa belladonna L. (n = 36) Belladonna,
Family: Solanaceae deadly nightshade

Belladonna has been extensively used in European medicine since the earliest times and is still regarded as one of the few indispensable drugs of plant origin. Because of the mydriatic action of the leaf juice when introduced into the eyes, the plant was frequently used by Italian and Spanish ladies as means of imparting a seductive appearance to their eyes.

The deadly nightshade is a native of Central and Southern Europe and Asia Minor. Nowea-days it is extensively cultivated as a medicinal crop in the United States. Europe and India. In India , it is found under cultivation chiefly in Kashmir and Chakrata (Uttar Pradesh). The plant which grows in the Himalayas and is also cultivated in Kashmir is not A. belladonna, but A.acuminata Royle differing in the leaf shape and the colour of flowers.

A. belladonna is a herbaceous perennial with a creeping rootstock, growing to a height of about 90-120cm and possessing alternately arranged ovate leaves and bell-shaped purplish flowers (yellow in Indian bella donna. A.acuminata). It bears shining brownish or black berries (Figure 15.1A).

The plants are raised from seeds sown in warm, shady places, not exposed to direct sunlight. Vegetative propagation through splitting of old rootstocks can be also practised. The soil should be a light, calcareous, well-drained loam containing decomposed humus and good quantities of minerals like potash and soda.

The leaves and tops are collected during the flowering season, when the alkaloidal concentration is greatest, varying from 0.9 to 1.23 per cent. The leaves (and fleshy roots, if used) are thoroughly dried, preferably with artificial heat for a few days and then pulverised.

All parts of the plant contain alkaloids but are more abundant in the physiologically active cells. A large number of alkaloids have been isolated which are collectively referred to as 'belladonna alkaloids'.

Atropine ($C_{17}H_{23}O_2N$), its isomer hyoscyamine and scopolamine ($C_{17}H_{21}O_4N$) are the three most used in medicines. Other related alkaloids, such as apoatropine, belladonine, noratropine, norhyoscyamine, tropacocaine and meteloidine are relatively unimportant therapeutically.

Atropine and hyoscyamine act as stimulants to the sympathetic nervous system and are employed as an antidote to opium. These are also used to reduce nasal secretions, sweating and also to control excessive salivation. Atropine has a stimulatory effect on the circulatory and respiratory systems. Internally, belladonna is used for the treatment of whooping cough and asthma and externally as a liniment to relieve neuralgic pain. Atropine is employed for dilation of the eye pupil during ophthalmologic examination and also to counteract muscle spasm (excessive muscular contraction).

Scopolamine, on the other hand, markedly depresses the parasympathetic nervous system thereby acting as a sedative or 'anti-insomniac'. It was formerly used with morphine to induce 'twilight sleep'.

The fruits are so poisonous that ingestion of three berries is sufficient to kill a child.

Besides *A.belladonna*, the leaves and seeds of other solanaceous plants such as jimson weed (*Datura stramonium* L.) and the leaves of the henbane plant (*Hyoscyamus niger* L.) contain atropine, hyoscyamine and scopolamine in varying proportions (Figures 15.1B-D). These solanaceous narcotics produce an intoxicating effect, when smoked or eaten, but continued used leads to disorders of the brain.

Cinchona spp. L.(x = 17) Fever bark tree
Family: Rubiaceae or quinine tree

The antimalarial property of Cinchona bark was known to the South American Indians from early times. Many stories are told regarding the discovery of *Cinchona*, revolving round the Countess of Chinchon, wife of the Viceroy of Peru, who was supposedly cured of malaria in 1638, after all other cures had failed, It is also learnt that the secret of the efficacy of this bark was revealed by a native maid out of affection for her mistress. The Countess was so pleased with the efficacy of the drug that she introduced it into Europe in 1640. In the eighteenth century, Linnaeus named it *Cinchona* in honour of this gracious lady. This often-quoted incident is, however, considered to be a myth. It is certain that Jesuit missionaries first brought it to Europe and its pharmaceutical value received recognition a few centuries later. The fame of the new remedy for malaria soon spread to other

countries and the Peruvian bark was in great demand. For over two centuries (from the mid seventeenth to the mid-nineteenth century), South America had a virtual monopoly of production. But due to reckless felling of the trees, it faced extinction that depleted the supplies in the Andean highlands.

Expeditions to collect seeds were sent out to the South American forests by the Dutch in 1852 and by the British government in 1859, in order to establish Cinchona plantations in South east Asia. In spite of strong vigilance of the forests by the native Indians, members of both the expeditions managed to smuggle seeds and seedlings out of South America. The seeds collected by the British expedition (under the leadership of Sir Clements Markham) became the basis of Cinchona succirubra Pav. plantations in India. Around 1861, Charles Ledger, an English resident in Bolivia, sent seeds of Cinchona to Europe. These seeds were collected by his faithful servant, who later died because of inhuman treatment by his compatriots for revealing the secret. Half of these seeds were taken by a planter to Ceylon and the remainder went to the Dutch Government. From Ledger's seeds, the Dutch established great plantations of C.ledgeriana Moen, in Java where climatic and topographical conditions were similar. Nearly 95 per cent of the world's export trade in quinine remained in the hands of the Dutch. All phases of Cinchona production, i.e. culture, harvesting processing and breeding were worked out, particularly in Java.

The Dutch venture flourished on a large scale until the outbreak of World War II. The capture of Java by the Japanese came as a serious blow to the United States and her allies. During the war, attention was again paid to the once rich South American jungles that were systematically exploited by North American technical expeditions. Several new sources were discovered, including the high yielding C.pitayensis Wedd. Latin America (especially Ecuador and Peru)became the chief producer between 1942 and 1945.

Before the discovery of antimalarial drugs, malaria was a common affiction in ancient times and was a constant obstacle to progress and development. It caused a great loss of human lives in Africa, Asia, the United States and also parts of Europe. Some scholars firmly believe that malaria was one of the factors which contributed tothe fall of Roman empire. It is even said that the ravages of malaria and the lack of adequate supplies of quinine played a decisive role inthe collapse of the German armies during World War 1.

The discovery of antimalarial drugs opened a new chapter in world history. The bulk of the commercial supply of the drug is derived from the bark of several species of Cinchona, a native to the Andean highlands of South America, extending from Bolivia to Colombia. The important species yielding the drug are: calisaya or yellow bark of commerce (C.calisaya Wedd.); ledger bark or ledger hybrid bark. C.ledgeriana Moens (= C. calisaya Wedd. var. ledgeriana Howard); loxa or pale bark ofcommerce (C.officinalis L.) and red bark of

commerce (*C.succirubra* Pay.). In addition to these, *C.micrantha* Ruiz. & Pav.and *C.nitida* Ruiz & Pav. of of Peru and Ecuador and *C.pitayensis* of Colombia also contain the alkaloid, *C. ledgeriana* gives the highest yeield of quinine, producing up to 16 per cent in the bark.

Cinchona spp. are evergreen shrubs or trees with opposite, simple entire leaves and interpetiolar stipules. Small fragrant flowers are borne on terminal panicles. The capsular fruits are oblong to ovoid-lanceolate (Figure 15.2).

The plants are found growing at altitudes ranging from 760 to 2750 m (seldom below 300 m), preferring cool mountain slopes with an abundant and well distributed rainfall of over 220 cm. The plants grow best on light, well-drained, virgin forest soils rich in organic matter, with a pH of 4.5 - 6.5 . Propagation is either by seeds or by vegetative means, i.e. grafting and cuttings.

At present, *Cinchona* is cultivated on a large scale in India and Indonesia. A limited supply is also obtained from Tanzania, Sri Lanka and Burma (Figure 15.3).

The bark of *Cinchona* roots contain the highest concentration of total alkaloids, but the bark of the trunk is the richest source of quinine. The harvesting of the bark may be undertaken from the fourth year onward but the alkaloidal content of the bark reaches its maximum only after 10-12 years of planning. Bark is removed by uprooting the trees when 12 years of age or

by cutting the stem above ground and then stripping the main stem and branches. The packed bark is subsequently shipped to the drug manufacturers.

Nearly 30 alkaloids have been isolated from Cinchona-spp. of which the most important are; quinine ($C_{20}H_{24}O_2N_2$), quinidine (isomer of quinine), cinchonine ($C_{19}H_{22}N_2O$) and its isomer cinchonidine - all four collectively known as 'totaquine'. The various alkaloids exist in the bark in combination with cinchotannic acid, quinic acid, free organic acids, tannins, colouring matters, gums, starch, vegetable matter and traces of volatile oils. Other less important alkaloids are; cinchotine, javanine, hydroquinine, hydroquinidine, cusconine, cusconidine, cuscamine and cuscamidine.

In addition to its use for the treatment of malaria fevers, it is also valuable as a tonic and an antiseptic. Quinine, quinidine and their compounds are employed in insecticides for the preservation of fur, feathers, wool, felt and textiles. The residual bark of Cinchona, left after the extraction of alkaloids, is used as a tanning material. The alkaloid quinidine is a cardiac depressant.

Several species of Remijia (family Rubiaceae), particularly R.purdieana Wedd. and R.pedunculata Flueck., are promising substitutes for Cinchona and contain substantial amounts of quinine and quinidine in the bark. Besides these, roots, stem and leaves of Dichroa febrifuga Lour. (Saxifragaceae) and leaves of Chamaebatia foliolosa Benth. (Rosaceae) are also sources of these alkaloids.

Cinchona has lost much of its importance through the widespread use of synthetic antimalarials free from some of the disadvantages of natural quinine. Plasmochin, quinachrine hydrochloride (atabrine), plasmoquine and chloroquine are prominent among the large number of compounds having anti-malarial properties.

Digitalis spl. Linn. (x = 7)   Foxglove
Family: Scrophulariaceae

Digitalis was used by the early herbalists for the treatment of epilepsy, wounds and other ailments. By using a leafy material obtained from the Witch Woman of Shropshire', William Withering succeeded in curing dropsy (unwanted accumulation of liquid in the body cavities)- now known to be a manifestation of heart disease. He published the first scientific account of foxglove and some of its medicinal virtues in 1785. But its true therapeutic value was recognised only in the earlier part of the twentieth century and it is now regarded as a pwerful medical agent controlling the myocardial tissue of the heart.

Thedrug is obtained from the dried leaves of the common foxglove, D.purpurea L., and wooly foxglove, D. lanata Ehrh., native of central and southern Europe. The plants are now cultivated on a commercial scale in the United States, Central Europe, England and Argentina. In India, D.purpurea is cultivated chiefly in Kashmir and the Nilgiri hills, while D.lanata is grown in Kashmir at altitudes of above 2100 m and also at Chakrata (Uttar Pradesh).

The plants are beautiful, biennial herbs with rosette-like leaves and are easily recognised by their pendant, zygomorphi, tubular; purple or yellow flowers each conspicuously spotted on the inner bottom surface of the tube (Figure 15.4). In D.lanata, the flowers are smaller, hairy and cream, yellow or purple coloured.

Leaves of D.purpurea are the largest and most commonly used. They are picked by hand. Collection made during the first year contain the highest percentage of glycosides. Harvesting should be accomplished before flowering and the leaves must be quickly and thoroughly dried at temperatures not exceeding 60°C. Sun dried leaves retain their activity for a longer period. Dried leaves after carefull packing are shipped for export.

The active constituents of digitalis are mainly confined to the epidermal and subepidermal collenchyma and the endodermal cells of the vascular bundles. The total concentration of different glycosides in the leaves is about one percent. It contains a number of chemically and physiologically related cardiac glycosides. The leaves also contain saponins, tannis, gallic, formic, acetic, lactic, succinic and benzoic acids and about 1.2 per cent of various saturated and unsaturated fatty acids.

The physiologically most active glycosides of the leaves of D.purpurea, namely digitoxin, gitoxin and gitalin are known to be derived from the naturally occuring purpurea glycoside A, purpurea glycoside B and purpurea glycoside C* respectively by the loss of a glucose residue. Digitoxin, gitoxin and gitalin,

on further hydrolysis, give rise to the non-sugar portion (aglycones or genins) digitoxigenin, gitoxigenin and gitaligenin respectively. It has been reported that the aglycones are weaker in cardiac action than the glycoside from which they are derived. Digitoxin is the most potent of the digitalis glycosides, its activity being 1000 times more than that of powdered digitalis. Digitalin is another active cardiac glycoside obtained from the seeds of D.purpurea.

D.lanata has stronger medicinal properties and its side effects are not as toxic as D.purpurea. The ac tive glycosides of the leaves are digitoxin, gitoxin and digoxin, being derived from the naturally occurring or primary glycosides, lanatoside A, lanatoside B and lanatoside C respectively (also known as digilands A, B and C). Lanatoside C has no counterpart in D. purpurea. On further hydrolysis, digitoxin, gitoxin and digoxin yield digitoxigenin, gitoxigenin and digoxigenin. Digoxin produces the same cardiac effect as digitalis, being 300 times more potent than that prepared from digitalis leaves.

Because of its stimulatory action on the heart, digitalis is often employed in the treatment of circulatory disorders. As it regulates the tone and rhythm of the heart beat, the contraction is made strong and regular. As a result more blood is sent out from the heart, thus helping in circulation . It is used as a myocardial stimulant in congested heart failure. It improves the blood supply to the kidnesy and, therefore, acts as a diuretic and removes renal obstruction.

Poisoning in human beings results from over-doses of the drug. Symptoms include nausea, diarrhoea, abdominal pain, gross disturbances in heart beat and pulse, various mental irregularities, drowsiness or or tremors and even convulsions.

Ephedra spp. (x = 7) Ephedrine
Family: Ephedraceae (Gymnospermae)

The therapeutical ac tion of Ephedra was known to the Chines more than 5000 years ago under the same 'Ma Huang', 'yellow hemp'. Even in the Vedic period some of its species are belived to have been used as divine plants. Although the alkaloids were isolated in 1885 by Yamanashi (ephedrine in pure form was isolated by N.Nagai in 1887), their pharmaceutical value remained obscure until 1923. It was through the work of Drs. K.K.Chen and C.F.Schmidt that the role of ephedrine as a sympathomimetic was discovered. The alkaloid ephedrine physiologically and chemically resembles epinephrine (adrenalin), a hormone-like substance having a stimulatory action on the sympathetic nervous system. Since the classical research of Chen and his associate, five species of Ephedra, namely E. sinica Stapf, E.equisetina Bunge, E. gerardiana Wall., E.major Host, and E.distachya L. have been found to be the chief sources of the drug Ephedra is grown on a large scale in China, India, Spain, U.S.A., Kenya and Australia.

The leafless plants have a shrubby appearance and resemble the common horsetails, occurring abundantly in arid regions (Figure 15.5).

The potent alkaloid ephedrine is extracted from the dried or fresh branches of the stem. It has been estimated that the alkaloidal concentration increases with the age of the plant and reaches its peak when plants are nearly four years old, still in flowering. Among the Indian species, E.major is the richest source of ephedrine and contains over 2.5 per cent of total alkaloids.

The major alkaloid ephedrine ($C_{10} H_{15} ON$) occurs in combination with several other alkaloids, such as d-and-l-pseudo-ephedrine (isoephedrine) and ephedine. Ephedrine, however, constitutes nearly three-quarters of the total alkaloids.

Zephrol containing ephedrine hydrochloride has been employed in the form of nasal drops to relieve nasal and sinus congestion and also for the treatment of bronchial coughs. The use of ephedrine preparations in asthma, hay fever and colds are very popular. It has been also used to control night wetting (urinating shile sleeping in children). When administered intravenously, it stimulates thesympathetic nervous system and counteracts, overdosages of depressants, thus causing an immediate and pronounced elevation in blood pressure.

Overdosages of ephedrine, however, cause headache, sweating, insomnia, nausea and vomiting.

At present a long list of synthetic derivatives of ephedrine are sold on the drug market, e.g. benzedrine, dexedrine and pervitin. They have been used for alertness and wakefulness by pilots, motorists and others whose occupation requires long hours of physical exertion.

They also seem to excite and impart thrills to their users, resulting in habituation.

Papaver somniferum Linn. (n = 11)

Opium poppy, white poppy

Family: Papaveraceae

Opium and its derivatives-particularly morphine and heroin are the most dangerous of the habit-forming narcotic drugs and are most difficult to give up. Taken in moderate quantities under the advice of a physician, they are legitimate remedies and afford relief to millions of sufferers. Opiates remain unexcelled as hypnotics and sedatives, and are freauently used to relieve pain and anxiety; they cause mental and physical relaxation and often induce badly needed sleep. At the same time, they have become a curse to mankind being smoked, eaten, sniffed or injected by untold millions of unfortunate addicts of all religions and nationalities all over the world. Addiction to opium and its derivatives is so consuming that people become slaves to the habit and may commit crimes to support it. The immediate effects are pleasurable, inducing dreams and visions. However, continued use of opium and its derivatives leads to physical, mental and moral degradation, the addict in due course becoming a victim of delirium and death.

Opium eating was once quite common in Persia (now Iran), Asia Minor and India and opium smoking in China and India. There are still a great many opium eaters and smokers in China, Egypt and India, where

opium is grown as a crop. Most drug addicts in the West today use heroin. Even today, opium dens are flourishing throughout the world, although opium consumption is illegal. In recent years opium addiction has greatly diminished owing to strict governmental control of the cultivation, distribution and export from the producing countries.

Crude commercial opium represents the air dried congealed or coagulated latex obtained from the unripe capsules of Papaver somniferum, believed to be a native of Asia Minor (Turkey) where it often grows as an escape. Opium was known to the ancient Greeks, Romans and Egyptians. It had reached Persia, India and China by the eighth century. In India, cultivation of poppy began during the early sixteenth century and was a profitable article of trade. The East India Company in 1767 started a flourishing business, exporting opium from India to China. The trade developed into such a great menace that the Chinese Government banned the import of the drug. The ban was one of the causes of the so-called 'opium war' of 1839-42 between Great Britain and China.

The opium poppy is also cultivated as an ornamental plant in many parts of the world. It is an erect, annual, glaucous herb, 30-100 cm tall. All plant parts contain latex. The leaves are ovate-oblong, with leaf bases embracing the stem, and are often shallowly pinnately lobed. The flowers are large and showy and are white or sometimes purple or scarlet in colour (Figure 15.6A). The fruit is a typical capsule developing from a multicarpellary ovary having parietal placentation.

The capsules are very large, ovoid or globular and topped by the persistent stigmatic disc with deep marginal lobes, dehiscing by means of valves or pores (Figure 15.6B). The seeds are small with a minute embryo in an oily endosperm.

In India, the opium poppy is cultivated as a rabi (winter) crop, the seeds being sown in October-November and the opium collected the following March-April. Opium poppy is grown in almost all types of soil but it prefers a well-drained sandy loam. The plant cannot tolerate extreme cold.

At present opium poppy plants are extensively cultivated in India. Turkey and U.S.S.R. A small amount is obtained from Yugoslavia, Bulgaria, Afghanistan, Pakistan and Japan. India is the only opium exporting nation. Opium poppy is cultivated in the states of Madhya Pradesh, Uttar Pradesh and Rajasthan.

To harvest the 'resin', incisions are carefully made into the maturing capsules from the bottom upwards with the help of specially designed tools. During lancing, deeper cuts are avoided so as to prevent the flow of milky latex among the seeds. The operation is carried out in the late hours of evening and before sunset. Exudate droplets collect on the surface of the capsule and are allowed to remain there overnight. The coagulated latex is scraped off before sunrise. Thick latex is stored in perforated metallic or earthen pots kept in a slightly tilted position to drain off water. Raw opium is then dried in the sun, which makes it viscid and dark brown, developing a characteristic odour. The thick mass is

kneaded by hand into balls, cakes or opium bricks and wrapped in poppy or Rumex leaves for further slow drying.

Crude opium contains a large number of alkaloids, about 35 in all, besides other components such as gum, rubber, resin, oils, moisture, pigments, meconic acid, and other extraneous matter. Among the most important alkaloids found are: morphine ($C_{17}H_{19}O_3N$), codeine ($C_{18}H_{21}O_3N$), thebaine ($C_{19}H_{21}O_3N$), papaverine ($C_{20}H_{21}O_4N$), noscapine, formerly called marcotine ($C_{22}H_{23}O_7N$), and narceine ($C_{21}H_{28}O_8N$).

Morphine is physiologically the most active of all the alkaloids; it was isolated in 1806 by Serturner. It is the principal alkaloid constituting 8-13 per cent of opium by weight. Because of its sleep and dream inducing properties, the alkaloid was named after Morpheus, the god of dreams. Morphine is essentially an analgesic and sedative. It is well known as a pain reliever. Heroin (diacetylmorphine) is a more powerful analgesic than morphine. A large number of new drugs with morphine-like activity have now been synthesised, prominent among which are; pethidine, dionin, metapon and the methadone group. Morphine is also used in a number of cough medicines, for allaying diarrhoea and vomiting, and to reduce blood pressure and bleeding. Codeine resembles morphine qualitatively, but is slightly milder in action as an analgesic. It is used in whooping cough medicines as codeine sulphate and codeine phosphate.

The seeds of the opium poppy are, however, free from the narcotic constituent and are used in food. They are quite nutritious and have a pleasant nutty

flavour and are often added to cakes or sprinkled on bread. The seeds are also a source of a fatty oil (poppy oil) which is used in India in the preparation of sweetmeats, curries, etc.

Rauvolfia serpentina Benth. ex Kurz. (n = 10, 11, 12, 22)
Rauwolfia
Family: Apocynaceae

The dried roots of Rauvolfia serpentina have long been used by the people of India as a cure for epilepsy, high blood pressure, insanity, intestinal disorders, cardiac diseases, snake-bite and as an anthelmintic. Some of the active alkaloidal constituents, e.g. ajmaline, ajmalinine, ajmalicine, serpentine and serpentinine were isolated for the first time by the Indian chemists Siddiqui and Siddiqui in 1931. Indian rauwolfias have now assumed a leading position in modern medicine, especially since the isolation of the main alkaloidal constituent, reserpine, by Muller, Schlittler and Bein of the CIBA Laboratories, Switzerland in 1952. Reserpine was the first tranquiliser to be used for the treatment of schizophrenia and other forms of mental disorders. Being a hypotensive agent, reserpine is widely employed today for hypertension (high blood pressure).

The drug rauwolfia is derived from the roots (especially the bark) of different species of Rauvolfia, named in honour of Leonhard Rauwolf, a German physician of the sixteenth century. Five species have been recorded in India, of which R.serpentina has attained a great reputation as a medicinal plant. R. tetraphylla L. and R.vomitoria Afzel. are two other species that are used

for extracting reserpine in America and Africa respectively. The reserpine content of R.vomitoria is, however, twic that of R.serpentina.

R.serpentina is an upright , perennating, evergreen, glabrous undershrub with tuberous roots with a characteristic slightly wrinkled and coarse surface. The root bark is greyish yellow to brown and displays irregular longitudinal fissures. The leaves are simple, glabrous, lanceolate or abovate and are generally in whorls of three to four, crowding the upper part of the stem. The inflorescene is generally terminal but sometimes axillary, and usually consists of dense crowded cymes (Figure 15.7).

R.serpentina grows wild in India, Bangaladesh, Sri Lanka, Burma, Thailand, Indonesia and Malaysia. The plants is found in almost all parts of India from Kerala to the Himalayan foothills, except Rajasthan province. It is widely distributed in the sub-Himalayan tract (up to an elevation of about 1000 m) and in the lower ranges of the easern and western ghats and in the Andamans. Collection from wild sources has decreased considerably in recent years. Experimental cultivation has been undertaken in many parts of the country such as Uttar Pradesh, Maharashtra, Bihar, Jammu and Kashmir, Tamil Nadu, Kerala, Central India and Gujarat.

The plant grows in tropical or sub-tropical regions, benefiting from the monsoon rains. It may be grown almost anywhere at low or medium elevation where rainfall is not less than 75 cm. Rauwolfias flourish in hot humid conditions and can be grown both in the open and in partial shade. Soils with plenty of humus and a pH 4.0-6.3, are desirable for good growth. The plants are

best raided from root-cuttings, but seeds and stem cuttings can also be used for propagation.

For commercial exploitation, roots are generally gathered two to three years after planting. It has been estimated that the alkaloidal content of the roots harvested after the shedding of leaves is far richer than the roots dug out in August. After harvesting, the roots are cleaned, air-dried and packed for shipment in airtight containers.

The total alkaloid content of the root varies from 1.7 to 3.0 percent, of which the bark alone accounts for nearly 90 per cent. The leaves and stems contain small amounts of alkaloids. A large number of alkaloids (80 or more) have been isolated from various species of Rauvolfia. Reserpine ($C_{33}H_{40}N_2O_9$) is pharmacologically the most potent. Other important alkaloids are; reserpinine, rescinnamine, deserpidine, deserpideine, serpentine, serpentinine, ajmaline, ajmalinine, ajmalicine, isoajmaline, rauwolfinine and yohimbine.

In addition to the uses of the drug mentioned earlier, it is known to stimulate uterine contraction and, therefore, is recommended for use in childbirth. An extract of the leaves has also been employed as a cure for the opacity of the cornea.

Strychnos nux-vomica L. (n = 12) Nux vomica

Family: Loganiaceae

The dried ripe seeds of Strychnos form the chief botanical source of the valuable alkaloids that have acquired prominence more as a poison than for medication. The use of Strychnos as a virulent arrow poison (curare) has been known since

antiquity. The medicinal and toxicological properties of the drug are mainly exhibited by the alkaloid strychnine.

Commercial supplies of the drug are obtained from S. nux-vomica, a native of southern Asia and Australia, and St. Ignatius's bean (S. ignatii Berg.) indigenous to the Philippine Islands. At present India, Sri Lanka, Malaysia, China and Australia are the chief producing countries. S. toxifera Schomb. ex Benth. and S. Castelnaei Wedd., are two well known sources of arrow poisons in South America.

The plants are woody vines or small trees which bear large berries resembling a mandarin or Chinese orange in shape and colour, each fruit containing from three to five greyish seeds (Figure 15.8). The seeds are hard, large circular or flattened structures having a bitter taste. The silky white sheen or lustre of the seeds is due to the presence of many closely appressed hairs (Figure 15.9).

Seeds are removed from the glutinous pulp either by washing or allowing the pulp to rot away. After a thorough washing and drying, the seeds are marketed. The seeds contain a high percentage of alkaloids (1.53-3.42 per cent) and are the principal source of the drug. Other plant parts such as old roots, wood, bark, leaves, blossom and the fruit pulp also contain varying amounts of alkaloids. The major alkaloids are strychnine and brucine. Besides these, the seeds also contain small amounts of strychnicine, vomicine, a-colubrine, B-colubrine and pseudostrychnine, which occur in combination with the glycoside loganin.

The drug is used for the treatment of nervous disorders and paralysis in minute dosages. Higher dosages are employed for destroying stray dogs and agricultural pests such as rats, rabbits, foxes, etc., the drug often being fed mixed with cereal flour. Strychnine and other alkaloids function as powerful stimulants on the central nervous system, especially of the spinal cord. Strychnine has been used as an antidote for barbiturate poisoning or other depressants. Since strychnine and brucine have a stimulating effect on the gastro-intestinal tract, the drug is often used as a tonic. Higher dosages of the drug produce muscular twitching or convulsions and, therefore, should be used with utmost caution.

Contd...P/27.

The fruit pulp is, however, non-poisonous and is eaten by birds, cattle and monkeys.

Claviceps purpurea (Fr.) Tul. — Ergot
Ascomycetes (group Hypocreales)

The dreaded illness 'ergotism'* caused by the prolonged consumption of ergotised rye bread, stimulated extensive research on the biochemical nature of the fruiting body, the sclerotium. Although the cause of the disease was known in 1597, the important alkaloidal constituent was isolated from the crude drug only in 1906. Even today, blighted rye grains are eaten by peasants faced with hunger.

Commercial ergot represents the dried fruiting body, the sclerotium, parasiting only the ovary of the developing flowers of *Secale cereale* L. and other grasses. The sclerotium is a purple, dark brown or black, curved cylindrical structure consisting of a pseudoparenchymatous mycelial mass rich in oil globules (Figure 15.10).

Ergot is a rich source of a number of pharmacologically active alkaloids - derivatives of either lysergic or isolysergic acids. Of the various alkaloids the best known physiologically active constituents are: ergotoxine (isolated by Berger and Carr in 1906), having an adrenolytic activity; ergotamine (A. Stoll in 1918), with a therapeutic use in obstetrics and ergonovine (Dudley and Moir in 1935), possessing the oxytocic principle. Today, all the naturally occurring alkaloids have been synthesised. In addition to the alkaloidal fraction, ergot contains sclererythrin (a red or violet pigment), ergosterol, clavicepsin, ergochrysin, ergoflavin, inorganic salts and large number of bases and amino acids.

The older term 'ergotoxine' found in the literature refers to an alkaloid complex consisting of three alkaloids (ergocryptine, ergocornine and ergocristine). Ergometerine and ergobasine are synonyms for ergonovine.

---

*Ingestion of smaller dosages of ergot everyday over a period of several weeks to a few months produces gangrenous ergotism (necrosis of tissues of the extremities) and convulsive ergotism.

Contd...P/28.

Owing to its stimulatory action on the muscular coats of the stomach, the intestine, the uterus and the blood vessels, ergot has been frequently used in obstetrics for accelerating childbirth. It is used extensively to increase the blood pressure (as a hypertensive agent) and also for controlling uterine haemorrhage after childbirth. Heavy dosages of ergot cause nausea, vomiting, diarrhoea, and sometimes lead to unconsciousness and collapse. Lysergic acid diethylamide (LSD), however, is one of the worst habit-forming drugs known, and is suspected of causing chromosomal aberrations (although unequivocal evidence has not yet been produced).

Commercial supplies of ergot comes chiefly from central Europe, Spain, Portugal and from North American sources.

ANTIBIOTICS

In the twentieth century, tremendous progress has been made in the development of new disease preventing agents - the antibiotics. These chemical substances are produced and excreted by the living organisms in the course of their metabolism and are capable of killing other organisms. These 'miracle drugs' are chiefly obtained from microorganisms.

The lowly moulds were employed as healers to relieve festering ulcers by the Chinese thousands of years ago. Also, several centuries ago, North American Indians are known to have used both soil and rotting woods for the treatment of would infections and healing festered cuts.

Pasteur and Joubert reported in 1887 that the growth of anthrax bacteria was slowed down by contaminating organisms. Again in 1889, Emmerich and Low revealed that a substance isolated from Pseudomonas aeruginosia (Schroeter) Migula had a similar inhibitory action. But the clinical importance of their work remained unrecognised for quite some time. It was only in 1928 that Sir Alexander Fleming, a bacteriologist working at St. Mary's Hospital, London, incidentally found that the green mould contaminating a culture of Staphylococcus caused lysis (dissolution and destruction) of adjacent bacterial colonies which were known to cause serious diseases in man (Figure 15.11).

Contd...P/29.

After careful experimental studies, he discovered that the lysis was due to a chemical substance produced by the fungus (Figure 15.12). The name 'penicillin', after the green mould Penicillium, was given to the drug which won him fame and recognition for his scientific contribution. The discovery of penicillin was the forerunner of the modern antibiotic age.

Fleming's early work on penicillin was continued by Dr. Howard Fiorey, Ernst Boris Chain, N. G. Heatly, and others at Oxford. They demonstrated the remarkable power of penicillin to control various animal diseases caused by several pathogens such as Staphylococci, Streptococci and Pneumococci. In 1941, the drug penicillin was first administered to human beings with spectacular results. Since that time many antibiotics have been developed.

The original penicillin was simply the filtrate of the nutrient broth upon which Penicillium notatum West. was grown. With the collaboration of scientists from the Northern Regional Research Laboratory of the United States Department of Agriculture, Florey, in 1942, succeeded in perfecting techniques for the mass production of penicillin. Selected strains of P. chrysogenum Thom are grown in small culture flasks containing standardised corn-steep liquor and lactose (soyabeam-meal for most actinomycetes). These cultures are, in turn, used to inoculate large 'seed tanks' which can be further employed to inoculate huge vats containing thousands of gallons of nutrient media or both. Sterile air is continuously bubbled through the mechanically agitated culture medium. After maximum growth of the fungus, the nutrient broth is filtered, subjected to solvent extraction from which penicillin is precipitated out by suitable techniques. Crystallisation, purification, standardisation and packaging are done quickly under aseptic conditions. The purified penicillin was found to be a thousand times more potent than Fleming's original material and was far more stable. By 1945, penicillin become available to almost all hospitals throughout the world. One part of pure penicillin in fifty million parts of water is sufficient to kill most microbes, which makes penicillin the physician's most potent weapon for curing many infectious diseases.

Contd...P/30.

Penicillin has also been artificially synthesised. So far, none of the other antibiotics has equalled penicillin and it is still the most powerful and least toxic antibacterial agent. Today, there are several types (at least a dozen) of penicillins, derived from strains of P. notatum and P. chrysogenum. Penicillin usually comes from the factory as the potassium or sodium salts of penicillin G (benzylpenicillin).

Today, about 800 or more antibiotics have been described; of these the penicillins, streptomycin, chloromycetin (chloramphenicol) and the tetracyclines have been widely used in medicine. Bacitracin and polymyxin are two of the relatively few antibiotics obtained from bacteria. Antibiotics have not been effective against most virus diseases. Isolation of jawaharine from Aspergillus niger van Tieghem (Mitra, 1968) created a sensation among research workers because this antitumour antibiotic was reported to have antiviral activity.

Apart from several microorganisms, antibiotics have been isolated from some of the algae, lichens, a number of flowering plants and also from animal sources. More than half of the antibiotics discovered are produced by antinomycetes. The available space in the text does not permit a detailed discussion of individual antibiotics. Only a few of them are, therefore, listed, along with data about their discovery.

Antibiotics are used extensively in veterinary medicine. They are used also in livestock feed as a supplement, in the treatment of plant diseases, and in the preservation of biological material.

Contd...P/31.

Table 1. Important antibiotics obtained from plant sources

Name of the antibiotic	Year	Discovered by	Source
Penicillin	1928	Alexander Fleming	Penicillium notatum West. P. chrysogenum Thom
Streptomycin	1943	Selman A. Waksmath et al.	Streptomyces griseus (Krainsky) Waksman & Henrici
Bacitracin	1945	H. Anker B. A. Johnson F. L. Meleney	Bacillus subtilis Cohn amend.
Chloromycetin or Chloramphenicol	1947	P. R. Burkholder	Streptomyces venezuelae Ehrlich, Gottlieb, Burkholder
Polymyxin	1947	Robert G. Benedict & A. F. Langlykke	Bacillus polymyxa (Prazmowski) Migula
Aureomycin	1948	B.M. Duggar et al.	Streptomyces aureofaciens Duggar
Neomycin	1948	Selman A. Waksman & H. A. Lechevalier	S. fradiae Waksman & Curtis
Hydroxystreptomycin	1950	Robert G. Benedict et al.	S. griseocarneus Benedict at al.
Terramycin	1950	A. C. Finley et al.	S. rimosus A. C. Finley et al.

Given Antib

Table 2. Some other important drug plants*

Name of the drug	Botanical name	Family	Place of origin	Part used	Active constituents	Uses
1	2	3	4	5	6	7
Aconite	Aconitum napellus L.	Ranunculaceae	Mountains of Europe and Western Asia	Dried roots	Aconitine, aconine, benzoylaconine	Externally used for neuralgia and rheumatism; internally to relieve pain and fever
Vasaka	Adhatoda uasica Nees.	Acanthaceae	India	Dried leaves	Vasicine, adhatodic acid, essential oil	Used as an expectorant, Component of glycodin vasaka - a cough mixture used in India
Wormseed	Artemisia maritima L.	Compositae	Pakistan	Dried immature leaves and flower heads	Santonin	Anthelmintic, more effective against round worm than threadworm; also used for dropsy
Senna	Cassia angustifolia Vahl (Indian senna)	Leguminosae	Somalia and Arabia	Dried leaves	Aloe-emodin, chrysophanic acid kampferin, sannoside A and B	Laxative used in habitual constipation
	C. acutifolia Del. (Alexandrian senna)	Leguminosae	Tropical Africa	Dried leaves		
Ipecac	Cephaelis ipecacuanha (Brot.) Rich.	Rubiaceae	Central and South America	Dried roots and rhizomes	Emetine, cephaeline	Used as expectorant and emetic; also in the treatment of amoebic dysentery and diarrhoea.

1	2	3	4	5	6	7
American-Wormseed	Cheropodium ambrosioides var. antrelminticum (L.) Gray.	Chenopo-diaceae	West Indies, and Central & South America	Fruits	Ascaridol	Anthelmintic-for hookworm infections
Licorice or liquorice	Glycyrrhiza glabra L.	Legumino-sae	Southern Europe and Middle East	Dried roots and rhizomes	Glycyrrhizin or glycyrrhizic acid (glycoside), glycyramarin, (bitter principle)	Used as an expectorant and demulcent: for flavouring candy and tobacco
Ginseng	Panax quinque-folia L.	Araliaceae	Northeastern U.S.A.	Dried roots	Panaquilon (glycoside)	Used as cure for almost all diseases in China
	P. ginseng C. A. Mey.	Araliaceae	Korea and Manchuria	Dried roots		
Psyllium	Plantago indica L.	Planta-ginaceae	Southern Asia	Dried husk and entire ripe seed	Xylin (mucilaginous content)	As cathartics - for the treatment of chronic constipation.
	P. ovata Forsk.					
Podophyl-lum	Podophyllum emodi Wall.	Berberi-daceae	India	Dried roots and rhizomes	Podophyllo-toxin, podophyllo-resin, picropodo-phyllin, podophyllic-acid	Used as an evacuant or purgative in cases of chronic constipati-on; also being used for tumorous growths
	P. peltatum L.		Eastern U.S.A.			

1	2	3	4	5	6	7
Cascara	Rhamnus purshiana DC.	Rhamnaceae	North America	Dried tree	Emodin, iso-emodin, aloe-emodin, chrysophanic acid	Tonic and Laxative
Rhubarb	Rheum officinale Baill.	Polygonaceae	China	Dried rhizome and root:	Emodin, iso-emodin, aloe-emodin, chrysophanic acid	Tonic and laxative
	R. palmatum L.					
Strophanthus	Strophanthus hispidus DC.	Apocynaceae	Tropical Africa	Seeds	Strophanthin-G (ouabain). strophanthin-K.	Cardiac or heart stimulant
	S. kombe Oliv.					
Chaulmoogra oil	Taraktogenos kurzii King (=Hydnocarpus kurzii (King) Wrtg.)	Flacourtiaceae	Burma and South eastern Asia	Seeds	Chaulmoogric acid, hydnocarpic acid, goric acid	Treatment of leprosy
Ashvagandha	Withania-somnifera Dural.	Solanaceae	India	Dried roots	Nicotine, somniferin, somniferinine, withananine	Diuretic; used in rheumatism and applied to ulcer carbuncles, and painful swellings.

* Betel, cocaine, hashish, ololiuqui and peyote are included in the chapter on fumitory and masticatory materials.

BUFFER SOLUTIONS

Dr.S.K.Mohapatra
Reader in Chemistry

What are buffer solutions?

Analytical processes involving separation, precipitation etc. required not only the knowledge of pH of the reaction medium but also its rigid maintenance during the course of changes. Many biological processes occur at specified pH. Enzymes have been found to be most active at a given pH value, known as the optimum pH for the particular reaction. (For most enzymes it is between 6 and 8). This requires the body fluids both extracellular and untracellular to be maintained rigidly within such pH limits. Experiments show that extracellular blood has pH 7.4 and intracellular blood has pH slightly lower than 7.0 These values are rigidly maintained inspite of addition of large amounts of acid in the form of hydrochloric, phosphoric, sulphuric and lactic acid during the normal process of metabolic activities. Carbon dioxide generated during oxidation of carbohydrates, fats and proteins dissolves in the bllod and forms carboric acid. How come then the blood is maintained at specific values? Co_2 is expelled through the lungs, and a part of other acids is excreted by the kidney as wine, while another part is neutralised by ammonia synthesized in the kidneys. But large fraction of the added acids or alkali (vegetation diets, fruits and drugs taken to gastric problems) is neutralised by what are known as buffer systems built in by nature.

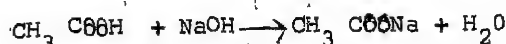
Experiments show that such systems consist of a mixture an weak acid or base and one of its salts, and they have the ability to resist change in pH when small amounts of acids and alkalis are added.

Mechanism of buffer action: The mechanism of buffer action can be explained by taking a buffer system as acetate buffer which is a mixture of acetic acid and sodium acetate. When an acid is added, the salt reacts with it to form acetic acid.



$$CH_3COONa + HCl \longrightarrow CH_3COOH + NaCl$$

and when an alkali is added, it reacts with the acid to form the salt.

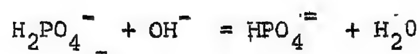


$$CH_3COOH + NaOH \longrightarrow CH_3COONa + H_2O$$

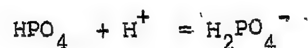
Thus the added acid is removed (neutralised) by the salt and the acid respectively. The acid formed does not dissociate appreciably in presence of the salt (common ion effect). Therefore the hydrogen ion concentration does not change or pH remains constant.

<u>Physiologically important buffers:</u>

Two-thirds of the buffer activity in vertebrates is carried out by phosphate buffer present in the kidney which is a mixture of KH_2PO_4 and Na_2HPO_4. KH_2PO_4 functions as the weak acid ($K_a = 6.2 \times 10^{-8}$) and Na_2HPO_4 functions as the salt and the reactions are

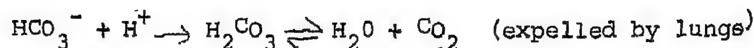


$$H_2PO_4^- + OH^- = HPO_4^{=} + H_2O$$

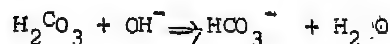


$$HPO_4^{=} + H^+ = H_2PO_4^-$$

The other third of the buffer activity is carried out by bicarbonate buffer which is a mixture of H_2CO_3 and HCO_3^- behaving as acid and salt respectively.

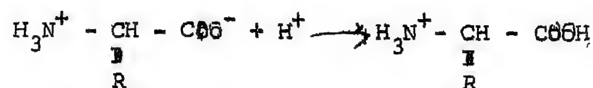


$$HCO_3^- + H^+ \longrightarrow H_2CO_3 \rightleftharpoons H_2O + CO_2 \quad \text{(expelled by lungs)}$$

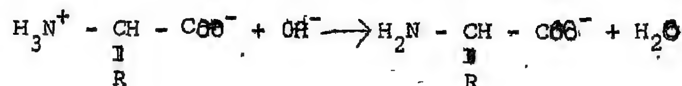


$$H_2CO_3 + OH^- \longrightarrow HCO_3^- + H_2O$$

In the tissues, the buffer activity is largely carried out by the plasma proteins. The amenoacids constituting the proteins can neutralize acids and alkalis as follows.

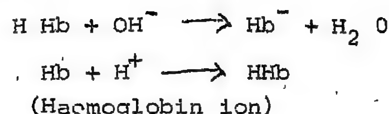


$$H_3N^+ - \underset{\displaystyle R}{\underset{|}{CH}} - COO^- + H^+ \longrightarrow H_3N^+ - \underset{\displaystyle R}{\underset{|}{CH}} - COOH$$



$$H_3N^+ - \underset{\displaystyle R}{\underset{|}{CH}} - COO^- + OH^- \longrightarrow H_2N - \underset{\displaystyle R}{\underset{|}{CH}} - COO^- + H_2O$$

Besides both deoxygenated and oxygenated haemoglobin which are polyprotic acids (having at least five acid groups) can also and do neutralise added acids and alkalis.



$$H\,Hb + OH^- \longrightarrow Hb^- + H_2O$$

$$Hb + H^+ \longrightarrow HHb$$

(Haemoglobin ion)

<u>Buffer range:</u>

Each buffer solution functions effectively within a small pH range called its range. The pH of a buffer solution is given by the Henderson equation.

$$pH = pK_a + \log \frac{[Salt]}{[Acid]} \text{ where}$$

$pK_a = -\log K_a$ (K_a being the dissociation constant of the acid). Therefore pH of a buffer solution depends upon (1) the dissociation constant of the acid and (2) ratio of the concentration of salt and acid. When the ratio is 10:1 $pH = pK_a + \log 10 = pK_a + a$ and when the ratio is 1:10, $pH = pK_a - 1$. When the ratio is greater than 10:1 or less than 1:10 the effectiveness of the buffer declines. Therefore the effective range is $pK_a \pm 1$. Thus acetate buffer has an effective range from 3.42 to 5.29 pK_a of acetic acid being 4.76 ; the phosphate buffer has a range 6 to 8 pK_a of KH_2PO_4 being 7.21.

<u>Preparation of Buffer Solutions:</u>

A buffer solution of a given strength and pH can be prepared as follows. Let us say we wish to prepare 0.2 M buffer of pH 5.4. First we have to choose an acid whose pK_a is around 5.4 . We refer to the dissociation constants of the acids. In this case we can either choose acetic acid (pK_a = 4 .76) or better monosodium - citrate (pK_a = 4.76). Next we have to find out the ratio of the concentration of the acid to the salt by using Henderson's equation

$$pH = pK_a + \log \frac{[Salt]}{[Acid]}$$

$$5.4 = 4.76 + \log \frac{[Salt]}{[Acid]}$$

Contd...4

Or $0.64 = \log\frac{[Salt]}{[Acid]} = \log\frac{[disodium\ citrate]}{[monosodium\ citrate]}$

Or $\frac{[Salt]}{[Acid]}$ = antilog of 0.64 = 4.365

Now the buffer to be prepared should be 0.2 M which means the total concentrated, i.e. Salt + Acid must be 0.2 M. Therefore if the concentration of Salt = x M, the concentration of the acid has to be (0.2 - X) M.

Hence $\frac{x}{0.2 - x} = 4.365$

Or $x = 0.8730 - 4.365x$ or $x(1 + 4.365) = 0.8730$

Or $x = \frac{0.8730}{5.365} = 0.1627$ M

Or [Salt] = 0.1627 M

and [Acid] = 0.2 - 0.1627 = 0.0373 M

Therefore the amount of salt to be taken is 0.1627 X 237 g (mol.wt. of disodium citrate) and amount of acid to be taken is 0.0373 X 214 g (mol wt. of monosodium citrate) and the volume made upto 1 litre.

Preparation of Buffer solutions of varying pH

Requirements:

1. Measuring flasks (500 ml) - 3
2. Measuring cylinders (400 ml, 50 ml. 25 ml) - 1 each
3. Reagent bottles - 9 nos.
4. Weighing bottle or glazed paper
5. Burette - (50 ml) - 1
6. Conical flasks (250 ml) - 6 nos.
7. Burette stand.
8. pH meter or Indicator soln.
9. Monosodium citrate
10. Disodium citrate
11. Sodium hydroxide
12. Chemical balance with weight box

Contd....5

Procedure:

(a) Prepare 500 ml each of 0.1 M solutions of monosodium and di-sodium citrate. Then prepare a series of buffer solution as follows.

Vol.of monosodium citrate soln.	Vol. of disodium citrate soln.
90 ml	10 ml
80 ml	20 ml
70 ml	30 ml
60 ml	40 ml
50 ml	50 ml
40 ml	60 ml
30 ml	70 ml
20 ml	80 ml
90 ml	10 ml

Measure their pH by any of the methods described in the previous unit and note in right column.

(b) Alternatively, take 25 ml of the monosodium citrate and neutralise to 10%, 20%, 30% ....to 90% by a standard alkali.

Preparation of some aqueous buffer solutions

(1) Acetate Buffer

(a) Acetic acid (0.2 M)

Dilute 28.5 ml of glacial acetic acid to 1 litre. This gives an approximately 0.5 M solution. Standardise this acid with 0.5 M NaOH solution and determine the exact strength. Then prepare an exactly 0.2 M solution of acetic acid by using the formula $V_1 \times S_1 = V_2 S_2$

(b) Sodium acetate (0.2 M)

Dissolve 16.4 g of sodium acetate in 1 l of distilled water.

Contd...6

Vol. of acetic acid	Vol. of sodium acetate soln.	pH
94	6	3.5
82	18	4.0
56	44	4.5
30	70	5.0
20	80	5.2
12	88	5.5
10	90	5.6

(2) Phosphate Buffer

(a) 0.025 M KH_2PO_4
Dissolve 3.4 g of KH_2PO_4 in 1l distilled wat.

(b) 0.025 M Na_2HPO_4
Dissolve 4.0 g of $Na_2HPO_4 \cdot H_20$ in 1 ltr. distilled water.

Equal volumes of (a) and (b) gives a buffer solution of pH 6.85.

Variation of pH in the range 5.85 to 7.8 can be made by varying amounts of the two solutions as in the case of acetate buffer pH can either be measured or calculated from Henderson's equation.

Note: The number of molecules of water of crystallization on the sample and take them into account in calculating molecular weight.

.....

INSTRUCTION IN PLANT PATHOLOGY

M.P. Sinha

A century agc a German dcctcr, Rcber, Kcch, became fascinated by millicns cf rcd-shaped bacteria in his micrcsccpe field. Cculd they be the cause cf the dreaded anthrax that was killing hundreds cf cattle and sheep thrcughcut Eurcpe. He dipped sharpened wccden splints in tc scme cf the fluid frcm diseased animals and carefully insected the splints intc his labcratcry mice. In the mcrning the mico were all dead, and in their blccd was the same kind cf rcd-shaped bacteria, prcviding evidence that these bacteria cculd indeed cause disease resembling anthrax. The prccedures which Dr. Kcch fcllcwed in prcviding evidence that bacteria assccıated with animals dead with anthrax were actually the cause cf that disease have beccme kncwn as Kcch's pcstulates cr Rules cf Prccf cf pathcgenicity. By these prccedures many diseases cf human and animals have been shcwn tc be caused by specific micrccrganisms. Likewise, they have been utilized rcutinely by plant pathclcgists tc prcve that bacteria and fungi cause disease in plants.

A plant becomes diseased where an interacticn between the plant and disease causing crganism (micrc-cr-ganism) results in the impairment cf the ncrmal physiclcgical functicns cf the plant. The severity cf the disease cr the extent cf the interacticn is a functicn cf the influence cf envircnment cn the develcpment cf the plant, cn the activities cf the micrccrganism and cn the interacticn between the plant and the micrccrganism. Disease in plants, therefcre, embcdies many biclcgical phencmenc. The fcllcwing steps may be crganised in crder tc study plant diseases.

1. Collecticn cf materials (diseased plants)

A walk arcund the vegetable cr flcwer garden, thrcugh the fields cn in the wccds during the grcwing seascn will reveal a variety cf spcts and blights cn plant leaves and stems (symptcms). Predcminants amcng these mcladies are mildews, the rusts, and the smuts, which are easily reccgnized by the vast numbers cf spcres prcduced cn the plant surface.

Make field trips in the late summer or late winter and observe plant foliage for mildews, rusts and smuts.Examine lesio in side with a hand lense.for the presence of hyaline chains of spores of the powdery mildews,for dusty orange or redish brown spores of the rusts or for brawnish-black masses of spores dust of the smuts.The whitish powdery mildews may be found in abundance on lilac,cucumber,grasses or legumes,Rusts abound on wheat,Oats,and grasses, an hollyhocks, and on some wild flowers. Smuts are less readily recognised by the casual observer but the common smut of corn may be found in nearly every field.

When you return to laboratory examine the specimens, microscopically,by cutting hand sections, staining in acid fuchin/ cotton blue for two minutes and mounting in glycerine. Press some leaf specimens and dry some smut galls for future use.

Expected Results:-The number of spores produced by these fungi is unbelievable. The powdery mildews derive their name from the"powdery" masses of spores piled on the leaf surface, In among these masses may be found the sexual fruiting bodies of this fungus,the perithe. filled with ascospores. These black structures have beautiful appendages,easily seen with the stereoscopic microscope, The rusts have variety of spores types, all produced by the hundreds in specialized fruiting structures. The black "dust" of the smuts, like-wise consists of hundred of tiny spores.

2. Isolation of plant pathogens(Bacteria and fungi)

Microorganisms suspected of causing disease in plants must first be isolated before pathogenecity can be demonstrated. The techniques used to isolate organisms vary with nature of the plant tissue invaded and the kind of microorganisms revolved. Many fungi and bacteria that cause disease in plants, may be isolated and grown niculture/pureculture in the class room

Procedure: a) Storage rot: Wash a fruit or vegetable,e.g apple or carrot,that is partially rotted. Immerse it in a dilute dis infestant(70% alcohol)for five minutes. Damp dry it with paper toweling and cut it open to expose the area between some rotted and healthy tissue. Transfer bit of tissue from this margin to PDA in petridishes,and space 4-5 pieces per plate. If examination for fungi only is desired,ard two drops of 50% lactic acid to the plate before

passing the agar. Mcre precise methcds fcr reccvery cf bacteria may be use,e.g. the serial dilutıcn technique.Incubate the dishes at rccm temperature.Start cbservaticn after 48 hrs.

b) Leaf and stem lesicns: Cc llect and wash spctted leaves cr stem and immerse 1.2mm squares cf tissue frcm the margin cf cf a diseased area in disnifestant.It may be necessary tc reduce the time cf immersicn cr strength cf disnifestant fcr this tissue tc avcid killing the causal crganisms.Transfer bits cf tissue tc P.D.A. and incubate at rccm temperature.

Observaticn:- Start cbservaticn after 48 hrs. fungi fcr bacteria present in diseased tissue will grcw cn tc the agar surface.Bacteria will be inhibited cn acidified PDA. If a particular cclcny type is reccvered ccnsistently frcm diseased tissue cf a certain plant it is circumstantıal evidence that the crganism may have caused the disease.

3. Preparaticn cf PDA(Pctatc dextrcse agar) media

Requirements

(a) Distilled cr tap water 1000 ml.
(b) Sliced unpeeled pctatc 200 gm.
(c) Agar 20 gm.
(d) Dextrcse 20 gm.

Prccedure.Ccck pctatces in 500 ml cf water fcr 20-30 minutes. This shculd be half bciled.Filter the brcth thrcugh clean cheese clcth cr crdinary clcth tc remcve the pctatc debris. Disscl ve the agar in ancther 200ml cf water. If necessary warm the water slightly. Disscl ve the dextrcse in ancther 200ml cf water. Mix the hct pctatc filtered brcth,the agar sclutic n and the dextrcse sclutic n in a 1000ml flask. Mix up thrcughly and add water tc make 1000 ml. Plug the flask with ncn-abxrbant cc ttcn and sterilize it in a pressure cccker fcr 30 minutes.

4. Stains:-

a) Acid fuchsion:- 0.5-1.00 gm cf acidfuchsin crystals tc be dissclve in water. If necessary may be warmed.

b) Cctton blue in lac tc phencl.

Lactcphencl sclution is prepared and ccnsists cf 20 gms phencl (warmed until melted), 2C ml lactic acid, 20 ml glycerin, 20ml distilled water. Tc cne-half cf the lactcphencl sclution is added encugh 0.5% cctton blue sclution in distilled water tc give a dark cclour.

CONCEPT AND CONCEPT TEACHING AND LEARNING AN OVERVIEW

Dr. S.C. Panda,
Lecturer,
RCE, Bhubaneswar.

What is a Concept:

A concept is a class of stimuli which have common characteristics. These stimuli are objects. events or persons. A concept is ordinarily designated by its name such as pencils, bottles, pupil or freedom fighters, committed workers, and nasty places etc. All these concepts refer to classes or categories of stimuli. But some stimuli do not refer to concepts i.e. Subhash Bose, Sarala Das (Adi Kabi), Tagore's Gitanjali, Indo-Pak war of 1971, Annual Book Exhibition. These are particular stimuli (not classes of), persons or events A concept is not a particular stimulus but a class of stimuli, The difference is between all freedom fighters and Subhash Bose. The concept freedom fighters includes Subhash Bose, but it includes many other fighters as well. The concept freedom fighters excludes all other war fighters. It is to be remembered that concept does not refer to particular stimuli but to classes of stimuli. The concept is a very broad one and it can include fighters of different types who faught for attaining freedom from British rule in various ways. Similarly, the concept bottles of varied sizes, colouration and shapes, pencils, of different lengths, qualities and types.

Hence concepts are not always congruent with our personal experience, but they represent human attempts to classify our experience at least crudely.

Contd.......2/-

Concept attributes:

An attributes is a distinctive feature of a concept and thus varies from concept to concept. For example: Red triangles which has two attributes: colour and form or shape. Colour can vary from concept to concept and, therefore, qualifies as an attribute. We can indeed have red squares, red rectangles, red trapiziums red parallelograms. A concept is lake.The chief attribute which distinguishes a lake from an ocean and sea,on one hand and from a pool and pond, on the other hand, in size. Size is one of its major attributes. Size qualifies as an attribute because it can vary from concept to concept. Of course there are other attributes of lake.

Attribute Values - Values are the particular variations an attribute may undergo. Colour is an attribute.It may have several values: red,white,blue,violet, black. Similarly form may have several values; rectangles squares, rohmbus,quadrangles. Concept vary in the number of values their attributes have. Some concepts have attributes with only two values. A student(a concept)can be a boy or girl, dead or alive, married or single.Other concept may have attributes with a range of value colour of an orange can vary from red-orange to yellow-orange. The colour,however,must not vary so much that we confuse an orange with a lemon or Mousumbi or shaddock. When an attribute has a wide range of values,the other attributes can be used to identify the concept in question. In identifying an orange the attributes of shape,size and texture can also be used.

Contd 3

Number of attributes:

The number of attributes varies from concept to concept. Red triangle : has only two attributes-colour and form. Small red triangle has three attributes-size, colour and form. An orange, has four attributes-colour, size, form and texture. Some complex concepts have a dozen or more attributes such as socialism, human rights, democracy etc. As the number of attributes increases the difficulty of learning of concept increases. Scanning the values of a dozen attributes is strenuous and time consuming. Bruner and his associates suggest that to have easy learning the number of attributes can be reduced by attending to some attributes and ignoring others or by combining a number of attributes into a smaller number of patterns.

Dominance of the Attributes:

Among the attributes physical location is more dominant than the attributes of colour and form. Also colour form concepts such as red triangles are more dominant than number-colour concepts such as one red. Thus, dominance refers to the concept as well as to its attributes. Dominant concept has dominant attributes. Learning concepts with dominant attributes with fewer examples is earlier than learning concepts with obscure attributes.

Informally, it is observed that students usually attend to certain points in their description of a concept but ignore other points that are equally important. Inplacing the concept of starrs children may attend to the attribute of placement/visibility of

Contd 4

celestial bright bodies in the night sky and ignore the condition of twinkling, movement, size, colour etc. Teachers must give aural or visual emphasis to attributes which are obscure and yet important in identifying the concept. In defining concepts teachers traditionally resort to vocal inflection, hand and arm gesticulation, underscoring, diagramming, drawing and so on, to make obscure attributes obvious or dominant. Unless this emphasis is provided, the student will learn some attributes and not others and, thereby, fail to learn the complete concept.

Types of Concepts:

Attributes combine in three different ways to produce three types of concepts: Conjunctive concepts, disjunctive concepts and relational concepts.

a) Conjuctive Concepts: The appropriate values of several attributes are jointly present. Ex-Three white half-shirts. It has three attributes (number, colour, form, joined together and each attribute has a particular value (respectively three, white, half-shirts). Conjuctive concepts are often the easiest to learn and to teach because of the additive quality of their attributes and values. Attributes and values are added together to produce a conjuctive concept. The student simply learn a list of attributes and appropriate values.

b) Disjunctive Concepts: It is the one that can be defined in a number of different ways. Attributes and values are substituted for one another.

Ex - a) Two figures and/or two circles.

b) Strike

c) Extra point in foot ball

Contd.....5

The attributes are form and number and the value of the number remains the same. The concept is disjunctive because the value of the form can change - it can be a circle or any form.

Disjunctive concepts are often difficult to learn because of the seemingly arbitrary equivalence of their attributes. Disjunctive concepts are, in effect, rules which the student must learn to apply to equivalent stimulus situations. But the situations are not equal or equivalent until given the level. Teachers must invest greater effort in the teaching of disjunctive concepts.

Relational Concepts: It is the one that has specifiable relationship between attributes.

Ex- Distance and direction are relational concepts. Distance specifies the relationship between two points; it refers to the separation of these points. Direction also specifies a relationship between two or more points; it refers to the movement from one to another point.

More examples - Time, many, few, average, longitude, mass, weight, mother, father etc. Relational concepts are more difficult to learn as the concept does not adhere in the attributes themselves. but in the particular relationships of the attributes. This sometimes creates lots of confusion in learning. For example, both the concept distance and the concept direction have as their attributes points in space and time. What distinguishes them is the difference in the relationship of the same attributes.

Contd.....6

What is Principle:

A principle is a statement of the relationship between two or more concepts. Principles are sometimes called rules or generalisations.

Ex- a) Rivers flow from hills to oceans.
 b) Thirteen minus four equals nine.
 c) The density of water is more than oil.
 d) Three dimentional objects have six sides

The following statements are not principles:

 a) Shyam likes Rahim
 b) Rajiv claims he is stronger than any body in India.
 c) Congress won the last election.
 d) Who is afraid of Nandan Kanan Tigers.

In the above set of statements concepts are there but those do not have relationships; basing on those concepts no rules or generalizatich can be made only the proper arrangement of the concepts results in satisfactory learning of principles.

When to teach concepts:

The teaching and learning of concepts must be related to the students' level of intellectual development. In teaching concepts during the period of concrete operations (age 7 to 11), the teacher must remember that the learner's thinking is oriented towards concrete objects in the immediate environment, that the child relinquishes the physical attributes of objects one by one, and that each grouping (or schema)remains an isolated organisation. In the period of formal operations,the adolescent child is capable of hypotheticodeductive and propositional thinking.

Contd.....7

Thinking. Although the teaching of concepts can and does occur during both periods, the teaching of principles proceeds more easily during the later period. Because the child's school learning of concepts is limited by his preschool learning, the school must often provide corrective experience to exclude irrelevant and include relevant attributes. Teacher should be in a position to decide on which concepts students should learn first and which they should learn later.

Educational uses of concepts and principles:

1) Concepts reduce the complexity of the environment.
2) Concepts help us to identify the objects of the world around us.
3) Concepts and principles reduce the necessity of constant learning.
4) Concepts and principles provide direction for instrumental activity.
5) Concepts and principles make instruction possible.
6) Concepts can be sterectypes. The teach r must Sometimes provide corrective experience for an additional use of concepts: Sterectypes. As concepts, sterectypes can sometimes be changed when the student is provided with a wider array of positive and negative examples then those which he has previously experienced.

The Teaching of Concepts

The teaching of concepts conform to the components of the basic teaching model. The process completes through seven steps steps 1 and 2 pertains to

Contd.....8/-

instructional objectives. Step 1 requires a statement of the objective, step 2, a type of task analysis. Step 3 provides the student with the appropriate entering behaviour. Step 4 through 6 are specific instructional procedures for concept teaching and step 7 deals with performance assessment.

Step 1 - Describe the performance expected of the student after he has learned the concept.

The expected performance is the correct identification of new examples of the concept. For the concept 'Satellites; the expected performance could be that when new examples of satellites given the learner will correct identify them. The description of terminal behaviour requires a performance quite different from rattling of definition. The point is that the description of the expected behaviour should not include the requirement that the student give a definition of the concept.

Describing terminal behaviour has two purposes. First the teacher has a means for assessing the adequacy of the performance and for determining the need for further instruction. The students' expected performance clearly indicates to the teacher and to the students the degree of adequacy the students are to attain a particular time. Second, the students have a way of assessing their own performance and of determining when learning is complete. The students' self-assessments then become a way of generating their own reinforcement.

Contd.....9/-

Step.2 Reduce the number of attributes to be learned in complex concept and make important attributes dominant.

In this step the values,number, dominance and relationship of attributes can be put to pedagogical use. The analysis of the concept is decided to teach.The determination of the values and number of attributes can be made before instruction is underway.The determination of dominance of the attributes requires experience and observation of important attributes students are likely to ignore. Then proceedures for teaching the concept are to be devised in two ways. Some of the attributes can be ignored and focus must be on those which the teacher thinks most important and/or the attributes can be coded into fewer patterns. But for a complete understanding of the concept,the learner would have to learn all the attributes listed with regard to a concept.

Step 3: Provide the student with useful verbal mediators

The teacher should ascertain the child's knowledge of the words used as attributes and attribute values and his knowledge of the relational words that are necessary. This step helps to see how the verbal and concept learning are related. The learning of certain names or labels (as verbal mediators) and specify type of verbal association facilities the students' learning of a concept.

Step 4: Provide positive and negative examples of the concept.

A positive example of a concept is one which contains the attributes of a concept. A negative example is one which does not contain one or mor more

Contd..... 10/-

256 of the attributes. Positive examples of the concept bird are crow, parrot, pegion, cuckoo, Negative examples are dog, cat, snake, fly, bat, bee. Use of positive and negative examples is a necessary condition for the learning of concepts.

The presentation of a mixed series of positive and negative examples is usually more effective than the presentation of a purely positive or a purely negative series. Presentation of only negative examples makes concept learning extremely difficult. As for number, enough positive examples to represent the range of attributes and attribute values of the concept should be presented. In the case of negative examples, at least, enough of these should be presented to eliminate irrelevant attributes which students are likely to include as part of the concept. Finally, direct experience or realistic examples are usually not preferable to simplified presentations of the concepts, such as line drawings, cartoons, diagrams and charts. These presentations help to achieve the effects of step 2, which directed to simplify the learning of the concept by focusing on its major attributes.

Steps 5: Present the examples in close succession or simultaneously

This step is concerned with the order in which the examples as a whole and the types of examples (positive and negative) are presented to the student. The learning condition is contiguity - the almost simultaneous presentation of the examples of the concept. Simultaneous presentation is better because the student does not have to rely upon memory or previous examples. In teaching the concept of dog,

Contd.....11/-

it is better to leave in view pictures of cats, birds, horses and dogs while presenting new pictures. By this miximization of contiguity and reduction of the information load on memory are taken care.

Step 6. Provide occasions for student responses and the reinforcement of these responses.

In concept learning reinforcement primarily provides informational feedback, which enables the learner either to separate positive and negative examples and to compose his list or to define the relationship of the various attributes. The primary purpose of reinforcement is to provide informational feedback to the student on the correctness of his responses. Since this feedback is crucial, any inconsistency, delay or failure to provide it will impair student learning. However, because the student knows which terminal behaviour he must acquire, he can to some extent monitor his own learning. Since reinforcement has motivational aspects, negative verbal feedback may impair concept learning by discouraging the student from making early guesses which can be confirmed. The teacher should remember to focus on the reinforcement of the students' responses and not on the student. The mode of the response should not be shifted, at least in the early learning of the concepts. It is quite possible, however, that the shift from spoken to written responses is less inhibiting than the shift from drawing to writing or writing to drawing.

Step 7: Assess the learning of the concept

In this step both contiguity and reinforcement are provided. This step emphasises generalisation, or the ability of the student to make the conceptual response to a new but similar pattern of stimuli. If the student is able to identify the new example of the concept, hea has learned the concept. Tp provide reinforcement the student must be informed about the accuracy of his response. Several new positive and negative examples of the concept are to be presented and the student has to select only the positive examples. A small amount of practice of the definition, even when the students are not told how good the definition is, improves the quality of definitions. When the definition is difficult to formulate special training for formulation of concept definition should be imparted.

Finalisation of Instructional objectives

S.C. Panda

The whole educational system is directed towards certain aims such as utilitarian,cultural,vocational,all round development of the learner and the like. The school education programme is only a part of the total educational programme.However,it plays an important and even a vital role in the realisation of educational aims.'What can the school education programme achieve?,'the question naturally arises. It can achieve only a part of these broad educational aims which we refer to an objective. An objective is a pointer an end-view of the possible achievement in terms of what a student is to be able to do when the whole educationalsystem is directed towards educational aims.

Objectives in Measurable Terms

On the other hand,educational objectives,bearing experiences and evaluation procedures are the three instructive aspects of the educational process.Objectives play a key role in the instructional process. They serve as guide for both teaching and evaluation. Instructional objectives determine precisely and specifically what type of pupil performance is desired at the end of the instructional sequence. Educational objectives have been stated as broad and ultimate goals such as exercising the mental functions of reasoning,imagination,and memory. The objectives should be stated in terms of students' behaviour that can be observed and measured. Armed ~~with~~ a clear and specific list of teaching objectives, a teacher may consider the most appropriate procedures for evaluating progress made towards each objectives. He attempts to test what he has tried to teach by using techniques best suited to determine how well each objective is attained.

Classification of Instructional objectives

The instructional objectives have been classified basing on three major domains: cognitive,affective and psychomotor. It was based upon the assumpation that in the process of sharing of how information changes usually occur in the domains of cognitive,affective and psychomotor of the learner.

The objective of the cognitive domain are phrased as descriptions of desired student behaviour - that is in terms of knowledge, understanding and abilities to be acquired. The large proportion of educational objectives fall into the cognitive domain. The affective domain includes objectives that emphasise interests, attitudes and values and the development of appreciation and adequate adjustment. Objectives in this domain are not stated very precisely and, infact, teachers do not appear to be very clear about the learning experiences which are appropriate to these objectives.

The psychomotor domain is concerned with physical, motor, or manipulative skills.

For further specifications of the taxonomy of educational objectives each of the three domains have been divided into a number of hierarchical categories of behaviours from simple to complex. For cognitive domain these six ascending levels are knowledge, comprehension, application, analysis, synthesis and evaluation. The five major categories of affective domain of the taxonomy of educational objectives are : receiving, responding, valuing, organisation, characterisation by a value or value complex. And finally seven major categories of psychomotor domain are: perception, set, guided response, mechanism, complex over response, adaptation and organisation.

While preparing instructional objectives it is possible to focus on different aspects of instruction. Our focus must on the behavioural changes(learning outcome) with the pupil which is based on the learning experiences provided to them.

<u>Criterion for selecting behavioural objectives</u>

In developing a list of objective for a particular course, however, the teacher is still faced with the problem of determining the adequacy of the final

list of objectives. The following list of questions will serve as a criteria for this purpose.

1. Do the objectives include all important outcome of the course?

2. Are the objectives in harmony with the general goals of schools?

3. Are the objectives in social principles of learning ?

4. Are the objectives realistic in terms of the abilities of pupils and the time and facilities available ?

General Instructional objectives and specific learning outcome

In preparing a list of instructional objectives for a course of study we have two immediate goals in mind. One is to obtain as complete a list of objectives as possible. This is most likely to occur if we follow the procedures for selecting objectives described earlier. The other goal is to state the objectives so that they clearly indicate the learning outcomes that we expect from our instruction. The task of stating instructional objectives is simplified if we constantly keep in mind that we are making a list of intended outcomes of teaching learning situation.

1) We are not identifying subject matter content but the reaction pupils are to make to this content.

2) We are not listing the learning experiences of the pupils but the changes in pupils performance resulting from these experiences.

3) We are not describing what we intend to do during instruction but are making a list of the expected results of that instruction. Stating objective in terms of learning outcomes rather than learning process admittedly is easier said than done. If we

continually ask curselves 'what shculd the pupils be able tc dc at the end cf the course cr unit of study, that they cculd nct dc at the begining. Then we find that the pupils terminal perfcrmance has almcst autcmatically become the center fccus. We are than in a much better positicn tc state cur instructicnal cbjectives in terms cf learning cutccmes.

A list cf cbjectives fcr a ccurse cr unit cf study shculd be detailed encugh tc clearly ccnvey the intend cf the instructicn and get general encugh tc serve as an effective cverall guide in planning fcr teaching and testing. This can be mcst easily accomplished by defining cbjectives in twc steps.

1) Stating the general cbjectives cf instructicn as intended learning cutccmes.

2) Listing under each cbjective a sample cf specific type cf perfcrmance that pupils are tc demcnstrate when they have achieved the cbjective. The prccedure wculd result in statements cf general instructicnal cbjectives and specific learning cutccme like the fcllcwing.

1. Understands scientific principles
   - 1.1 Describes the principles in his cwn wcrds.
   - 1.2 Identifies examples cf the principle
   - 1.3 States tanable hypcthesis based cn the principles
   - 1.4 Distinguish between twc given principles.
   - 1.5 Explain the relaticnship between twc given principles.

It is tc be ncted that the general cbjective starts right cff with verb with precise wcrding directing tc students cutccme and free cf ccurse ccntent. It shculd be unitary and realistic. Similarly it shculd be ncted that specific learning cutccme cr specificaticn is merely a sample cf the many specific ways tc realise the general

objectives. In case of specification to each statement should begin with a verb indicating observable responses. The specific learning outcomes are free of course content, realistic, unitary and stated in precise terms. Action verb is a key element in stating the specific learning outcomes the selection and clarification of these verbs play an important role in obtaining a clearly defined set of instructional objectives. Ideally we would like each verb

(i) to clearly convey our instructional intent and

(ii) to precisely specify the pupil performance we are willing to accept as evidence that the general objectives has been attained. Unfortunately some verbs convey instructional intent well (e.g. identifies), other are more effective at precisely specififying the pupil responses to be observed (e.g. encircles, lables, underlines). Where it is necessary to choose between two types it would seen desirable to select than that most clearly convey instructional intent and if needed, to further clarify the expected pupil responses in one of the following ways.

(a) Add a third level of a specificity to the list of objectives. E.g.

1. Comprehend the meaning of written material
   1.1 Identifies the main thought in a passage
      1.1.1 Underlines the topic/sentence
      1.1.2 Selects the most appropriate title for the passage.
      1.1.3 Writes the main idea of the passage.

(b) Provide definitions of the action verb used in the specific learning outcomes. E.g.
Illustrations of how to clarify expected pupils responses for selected action verbs.

* Actionverb	Types of responses
Identify	Point to, touch, mark encircle, match, pick up.
Name	supply verbal label(orally or in writing)
Describe	supply a verbal account(orally or in writing)that gives the essential categories, properties and relationship.

Actionverb	Types of responses
Order	list in order, place in sequence, arrange, rearrange.
Construct	Draw, make design, assemble, prepare, build.
Demonstrate	perform a set of procedure with or without, a verbal explanation.

(c) Use sample test items to illustrate the intended outcomes.

* Sullivan, H.J. (1969) stated that these six action verbs and their synonyms encompass all cognitive learning outcomes in the school.

Summary of steps for stating Instructional Objectives

The final list of objectives for a course, or unit should include all important learning outcomes(e.g. knowledge, understanding, skills, attitude, and should be stated in a manner that clearly conveys what pupils are like at the end of the learning experience. The following summary of steps provides guidelines for obtaining a clear statement of instructional objectives.

I. Stating the General Instructional objectives

1. State each general objective as an intended learning outcome (e.g. pupils terminal performance)

2. Begin each general objective with a verb (e.g. knows, applies, interprets) "omit" the pupil should be able to ....

3. State each general objective to include only one general learning outcome (e.g. not knows and understands).

4. State each general objective at the proper level of generality (i.e. it should encompass a readily definable domain of responses) stating from eight to twelve general objectives will usually suffice.

5. Keep each general objective sufficiently free of course content so that it can be used with various units of study.

6. State each general objective so that there is minimum overlap with other objectives.

II. Stating the specific learning Outcomes

1. List beneath each general instructional objective a representative sample of specific learning outcomes that describes the terminal performance pupils are expected to demonstrate.

2. Begin each specific learning outcome with an active verb that specifies observable performance (e.g. identifies, describes).

3. Check to be sent that each specific learning outcome is relevant to general objective it describes.

4. Include a sufficient number of specific learning outcomes to describe adequately the performance of pupils who have attained the objectives.

5. Keep the specific learning outcomes sufficiently free from course content so that the list can be used for other units of the study.

6. Consult reference materials for the specific components of those complex outcomes that are difficult to define (e.g. critical thinking, scientific attitude, creativity).

7. Add a third level of specificity to the list of outcomes it needed.

Table - 1

Major Categories in the Cognitive Domain of the Taxonomy of Educational Objective(Bloom,1956).

Descriptions of the Major Categories in the Cognitive Domain
1. Knowledge. Knowledge is defined as the remembering of previously learned material. This may involve the recall of a wide range of material, from specific facts to complete theories, but all that is required is the bringing to mind of the appropriate information. Knowledge represents the lowest level of learning outcomes in the cognitive domain.
2. Comprehension. Comprehension is defined as the ability to grasp the meaning of material. This may be shown by translating material from one form to another (words of numbers), by interpreting material (explaining or summarizing), and by estimating future trends (predicting consequences or effects). These learning outcomes go one step beyond the simple remembering of material, and represent the lowest level of understanding.
3. Application. Application refers to the ability to use learned material in new and concrete situations. This may include the application of such things as rules, methods, concepts, principles, laws, and theories. Learning outcomes in this area require a higher level of understanding than those under comprehension.
4. Analysis. Analysis refers to the ability to break down material into its component parts so that its organizational structure may be understood. This may include the identification of the parts, analysis of the relationships between parts, and recognition of the organizational principles involved. Learning outcomes here represent a higher intellectual level than comprehension and application because they require an understanding of both the content and the structural form of the material.

5. Synthesis. Synthesis refers to the ability to put parts together to form a new whole. This may involve the production of a unique communication (theme or speech), a plan of operations (research proposal), or a set of abstract relations (scheme for classifying information). Learning outcomes in this area stress creative behaviours, with major emphasis on the formulation of new patterns or structures.

6. Evaluation. Evaluation is concerned with the ability to judge the value of material (statement, novel, poem, research report) for a given purpose. The judgement are to be based on definite criteria. These may be internal criteria (organization) or external criteria (relevance to the purpose) and the student may determine the criteria or be given them. learning outcomes in this area are highest in the cognitive hierarchy because they contain elements of all of the other categories, plus value judgements based on clearly defined criteria.

Examples of General Instructional Objectives and Clarifying Verbs for the Cognitive Domain of the Taxonomy

Illustrative General Instructional Objectives	Illustrative Verbs for Stating specific Learning Outcomes
Knows common terms Knows specific facts Knows methods & Procedures knows basic concepts. Knows principles.	Defines, describes, identifies, labels, lists, matches, names, outlines, reproduces, selects, states.
Understand facts & Principles. Interprets verbal material Interprets charts and graphs Translates verbal material to mathematical formulas. Estimates consequences implied in data justifies methods and procedures.	Converts, defends, distinguishes, estimates, explains, extends, generalizes, gives examples, infers, paraphrases, predicts, rewrites, summarizes.

Applies principles to new situations. Applies theories to practical situation. Solve mathematical Problems. Constructs charts and graphs. Demonstrates correct usage of a procedure.	Changes, computes, demonstrates, discovers, manipulates, modifies, operates, predicts, pre-pares, produces, relates, shows, solves, uses.
Recognizes unstated assumptions. Recognizes logical fallacies is reasoning Distinguishes between facts and inferences Evaluates the relevancy of data Analyzes the organizational structure of a work (art, music, writing)	Breaks down, diagrams, differentiates, discriminates, distinguishes, identifies, illustrates, infers, outlines, points out, relates, selects, separates, sub-divides.
Writes a well-organized theme. Gives a well-organized speed. Writes a creative short story (or poem) proposes a plan for an experiment Integrates learning from different areas into a plan for solving a problem Formulates a new scheme for classifying objects (or events or ideas)	Categorizes, combines, compilies, composes, creates, devises, designs, explains, generates, modifies, organizes, plans, rearranges, reconstructs, relates, reorganizes, revises, rewrites, summarizes, tells, writes.

Judges the consistency of written material. Judges the adequacy with which conclusions are supported by data. Judges the value of a work (art, music, writing) by use of internal criteria Judges the value of a work (art, music, writing) by use of external standards.	Appraise, compares, concludes, contrasts, criticizes, describes, discriminates, explains, justifies, interprets, relates, summarizes, supports.

Table -2

Major Categories in the Affective Domain of the Taxonomy of Educational Objectives (Krathwo, 1964).

Description of the Major Categories in the Affective Domain

1. Receiving. Receiving refers to the student's willingness to attend to particular phenomena or stimuli (classroom activities textbook, music, etc.). From a teaching standpoint, it is concerned with getting, holding, and directing, the student's attention. Learning outcomes in this area range from the simple awareness that a thing exists to selective attention on the part of the learner. Receiving represents the lowest level of learning outcomes in the affective domain.

2. Responding. Responding refers to active participation on the part of the student. At this level he not only attends to a particular phenomenon but also reacts to it in some way. Learning outcomes in this area may emphasize acquiescence in responding (reads assigned material), willingness to respond (voluntarily reads beyond assignment), or satisfaction in responding (reads for pleasure or enjoyment). The higher levels of this category include those instructional objectives that are commonly classfied under interest; that is those that stress the seeking out and enjoyment of particular activities.

3. Valuing. Valuing is concerned with the worth or value a student attaches to a particular object, phenomenon, or behaviour. This ranges in degree from the more simple acceptence of a value (desires to improve group skills) to the more complex level of commitment (assumes responsibility for the effective functioning of the group). Valuing is based on the internalization of a set of specified values, but clues to these values are expressed in the student's overt behaviour. Learning outcomes in this area are concerned with behaviour that is consistent and stable enough to make the value clearly identfiable. Instructional objectives that are commonly classified under attitudes and appreciation would fall into this category.

---

4. Organization. Organization is concerned with bringing together different values, resolving conflicts between them, and beginning the building of an internally consistent value system. Thus the emphasis is on comparing, relating, and synthesizing values. Learning outcomes may be concerned with the conceptualization of a value (recognizes the responsibility of each individual for improving human relations) or with the organization of a value system (develops a vocational plan that satisfies his need for both economic security and social service). Instructional objectives relating to the development of a philosophy of life would fall into this category.

---

5. Characterization by a value complex. At this level of the affective domain, the individual has a value system that has controlled his behaviour for a sufficiently long time for him to have developed a characteristic life style. Thus the behaviour is pervasive, consistent and predictable. Learning outcomes at this level cover a broad range of activities but the major emphasis is on the fact that the behaviour is typical or characteristic of the student. Instructional objectives that are concerned with the student's general patterns of adjustment (personal, social, emotional) would be appropriate here.

Examples of General Instructional Objectives and Clarifying Verbs for the Affective Domain of the Taxonomy

Illustrative General Instructional Objective	Illustrative Verbs for stating Specific Learning Outcomes
Listens attentively Shows awareness of the importance of learning Shows sensitivity to social problems Accepts differences of race and culture Attends closely to the classroom activities.	Asks,chooses,describes, follows,gives,holds, identifies,locates,names, points to,selects,sits erect,replies, uses.
Completes assigned home-/ Obeys school rules Participate in class discussion Completes Laboratory work Volunteers for special tasks shows interest in Enjoys helping others.	Answers,assists,complies, conforms,discusses, greets, helps, labels,performs practices, presents, reads, recites,reports, selects, tells,writes.
Demonstrates belief in / the democratic process. Appreciates good literature(art or music),Appreciates the role of science(or other subjects) in everyday life shows concern for the welfare of others.Demonstrates Problem-solving attitude Demonstrates commitment to social improvement.	Completes,describes,differentiates,explains,follows, forms,initiates,invites, joins,justifies,proposes, reads,reports,selects, shares,studies,works

Recognizes the need for balance between freedom and responsibility in a democracy, Recognizes the role of systematic planning in solving problem. Accepts responsibility for own behaviour. Understands and accepts own strengths and limitations. Formulates a life plan in harmony with his abilities interests, and beliefs.	Adheres, alters, arranges, Combines, compares, completes, defends, explains, generalizes, identifies, integrates, modifies, orders, organizes, prepares, relates, synthesizes,
Displays safety consciousness. Demonstrates self-reliance in working independently. Practices Cooperation in group activities. Uses objective approach in problem solving. Demonstrates industry and self-discipline Maintains good health habits.	Acts, discriminates, displays, influences, listens, modifies performs, practices, process, qualifies, questions, revises, serves, solves, uses, verities.

Table -3

A Classification of Educational Objectives in the Psychomotor Domain (Simpson, 1972).

Description of the Major Categories in the Psychomotor Domain
1. Perception. The first level is concerned with the use of the sense organs to obtain cues that guide motor ac-tivity. This category ranges from sensory stimulation (awareness of a stimulus), through cue selection (selecting task-relevant cues), to translation (relating cue perception to action in a performance).
2. Set. Set refers to readiness to take a particular type of action. This category includes mental set (mental readiness to act), physical set (Physical readiness to act) and emotional set (willingness to acat). Perception of cues serves as an important prerequisite for this level.

3. Guided Response. Guided response is concerned with the early sages in learning a complex skill. It includes imitation (repeating an acat demonstrated by the instructor) and trial and error (using a multiple-response approach to identify an appropriate response). Adequacy of performance is judged by an instructor or by a suitable set of criteria.

---

4. Mechanism. Mechanism is concerned with performance acts where the learned responses have become habitual and the movements can be performed with some confidence and proficiency.Learning outcomes at this level are concerned with performance skills of various types,but the movement patterns are less complex than at the next higher level.

---

5. Complex Overt Repcnse.—Complex Overt Response is concerned with the skillful performance of motor acts that involve complex movement patterns. Proficiency is indicated by a quick,smooth,accurate performance, requiring a minimum of energy.This category includes resolution of uncertainity (performs without hesitation) and automatic performance (movements are made with ease and good music control). Learning outcome at this level include highly coordinated motor activities.

---

6. Adaptation.Adaptation is concerned with skills that are so well developed that the individual can modify movement patterns to fit special requirement or to meet a problem situation.

---

7. Origination. Origination refers to the creating of new movement patterns to fit a particular situation or specific problem. Learning outcomes at this level emphasize creativity based upon highly developed skills.

Example of General Instructional Objectives and Clarifying Verbs for the psychomotor Domain.

Illustrative General Instructional Objectives	Illustrative Verbs for Stating Specific Learning outcomes
Recognizes malfunction by sound of machine. Relates taste of food to need for seasoning Relates music to a particular dance step.	Chooses, describes, detects, differentiates, distinguishes, identifies, isolates, relates, selects, separates.
Knows sequence of steps in Varnishing wood Demonstrates proper bodily stance for batting a ball Shows desire to type efficiently.	Begins, displays, explains, moves, proceeds, reacts, responds, shows, starts, volunteers.
Performs a golf swing as demonstrated Applies first aid bandage as demonstrated Determines best sequence for preparing a meal.	Assembles, builds, calibrates, constructs, dismantles, displays, disects, fastens, fixes, grinds, heats, manipulates, measure, mends mixes, organizes, sketches.
Writes smoothly and legibly Sets up laboratory equipment Operates a slide projector Demonstrates a simple dance step.	(Same list as for Guided Response).
Operates a power saw skillfully Demonstrates correct form in swimming Demonstrates skill in driving an automobile Performs skillfully on the Violin Repairs electronic equipment quickly and accurately.	(Same list as for Guided Response)
Adjusts tennis play to counteract opponent's style Modifies swimming strokes to fit the roughness of the water.	Adapts, alters, changes, rearranges, reorganizes, revises, varies.
Creates a dance step Creates a musical composition Designs a new dress style.	Arranges, combines, composes, constructs, creates, designs, originates.

List of Resource persons
and Supporting staff

Resource Persons

1. Dr.N.Khattar
Professor and Head
Department of Science&
Programme Director
R.C.E.,Bhubaneswar

2. Dr.A.L.N. Sarma
Reader in Zoology
R.C.E.,Bhubaneswar

3. Dr.S.K. Mohapatra
Reader in Chemistry

4. Dr.M.P.Sinha
Reader in Botany
R.C.E.,Bhubaneswar

5. Dr.P.K. Durani
Reader in Botany
R.C.E.,Bhubaneswar

6. Dr.U.K. Nanda
Reader in Zoology&
Programme Coordinator
R.C.E.,Bhubaneswar

7. Dr.L.G. Rao
Sr.Lecturer in Zoology
R.C.E.,Bhubaneswar

8. Dr.Miss R.Mishra
Sr. Lecturer in Botany
R.C.E.,Bhubaneswar

9. Sri P.K. Patnaik
Lecturer in Zoology
R.C.E.,Bhubaneswar

10. Dr.G.V. Gopal
Lecturer in Botany
R.C.E.,Bhubaneswar

11. Dr.Miss Geetha G.Nair
Lecturer in Botany
R.C.E., Bhubaneswar

12. Dr.S.C. Panda
Lecturer in Education
R.C.E.,Bhubaneswar

13. Mrs.P.Mishra
Lecturer in Life Science
R.C.E.,Bhubaneswar

14. Dr.J.K. Panda
Lecturer in Zoology
R.C.E.,Bhubaneswar

External Resource Persons

15. Dr.S.D. Tripathy
Director, CIFA
Kaushalyaganga
Bhubaneswar

16. Dr.S.N. Patnaik
Professor in Botany
Utkal University
Bhubaneswar

17. Dr. J.Mohapatra
Medicine Specialist
Sahid Nagar,Bhubaneswar

Supporting Staff

1. Sri S.K. Acharya
Steno to Professor
and Head of Science
R.C.E., Bhubaneswar

2. Sri K.N. Patra
Lab.Assistant
R.C.E., Bhubaneswar

3. Miss N.Kanungo
Lab. Assistant
R.C.E., Bhubaneswar

4. Sri L.D. Samantaray
L.D.C. R.C.E.,Bhubaneswar

List of Participants

1. Sri Ramkrishna Chourasia
   Teacher Grade II
   S.E.Railway
   Mixed Higher Secondary
   School, Nainpur, Mandla
   Madhya Pradesh

2. Sri Tulsi Prasad Das
   Sr. Teacher GradeI(Biol)
   Chittaranjan High School
   Dist: Burdwan(West Bengal)

3. Sri Krishna Chandra Verma
   Sr. Teacher Grade I
   D.V.(Girls) Chittaranjan
   At/Po:Chittaranjan(W.B)

4. Mrs. Prem Kanwar Rathore
   Rly.Sr.Higher Secondary
   School, Bamdikui (Rajasthan)

5. Sri Raghuraj Prasad Tiwari
   P.G.T.(Biology)
   D.L.W. Inter College
   Varanasi(Uttar Pradesh)

6. Sri V.S. Chaubey
   Lecturer
   E.Rly Inter College
   Mugalsarai(Uttar Pradesh)

7. Sri K.Lodge
   Lecturer
   SE Rly.Mixed Higher
   Secondary School, Bhillai
   Marshelling, M.P.

8. Sri SK Borhan Ali
   Rly.H.S. School
   PO: Badarpur (Assam)

9. Sri Chandra Bhan Yadav
   Chittaranjan High School
   (E.M.)H.S.
   AT/PO: Chittaranjan
   Dist: Burdwan (W.B.)

10. Sri Monmatha Nath Khatua
    Deshabandhu Vidyalaya(Girls)
    PO: Chittaranjan
    Dist: Burdwan (W.B)

11. Sri S.S. Srivastava
    N.E.Rly Boy's Inter
    College(Hindi Medium)
    Gorakhpur(U.P)

12. Sri R.P.B. Singh
    P.G.T.(Biology)
    N.E.Rly.Senior Secondary
    School, Gorakhpur(U.P.)

13. Sri Mani Bhushan Prasad
    Lecturer (Biology)
    N.E.Rly Inter College
    Garahara(Barauni)Bihar

14. Sri B.S. Mukherji
    P.G.T.(Biology)
    SER/MHSS/EM
    Bilaspur, Madhya Pradesh

15. Kum.Anuradha Chatterjee
    P.G.T.(Biology)
    SER/MHSS/HM, Bilaspur
    Madhya Pradesh

16. Kum.Sarita Bangre
    P.G.T.(Biology)
    Central Railway Higher
    Secondary School
    New Katni Junction(M.P)

17. Sukla Chakraborty
    P.G.T.(Biology)
    Bani Mandir Rly H.S.School
    Siliguri, Darjeling(W.B.)

18. Smriti Bhattacharjee
    P.G.T.(Biology)
    Netaji Vidyapeeth Rly H.S.
    Secondary School, Maligaon
    Gauhati(Assam)

19. Miss Chitra Roy
    P.G.T.(Biology)
    Alipurlura J.N.Railway
    Higher Secondary School
    PO: Alipurduar Junction
    Dt: Jalpaiguri(W.B.)

20. Sri Dipak Mukherjee
    Teacher Gr.I(Biology)
    N.E.Rly.H.S. School.
    Alipurdura Junction
    West Bengal

21. Sri Mahavir Singh
    Senior Teacher
    W.Rly Hr.Sec. School
    Ratlam, Madhya Pradesh

22. Sri Karunakar Rath
    P.G.T.(Zoology)
    SE Rly.M.H.S. School
    Khurda Road, Orissa

23. Sri B.Subrahmonyam
    P.G.T.(Bio.Science)
    S.E.Rly.M.H.S. School
    (English Medium)
    Kharagpur(West Bengal)

24. Sri Bimal Prasad Mohanty
    P.G.T.(Botany)
    S.E.Rly.M.H.S. School
    Khurda(Orissa)

25. Sri K.Shyam Sunder Rao
    P.G.T.(Bio-Science)
    S.E.Rly.Boys' H.S. School
    Kharagpur, Midnapore(W.B.)